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#7 Appeal brief (3)

(file in triplicate)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: CLASSEN, John B.)
Serial No.: 08/591,651)
Filed: February 12, 1996)
For: METHOD AND COMPOSITION FOR AN EARLY VACCINE...)

Art Unit: 1643
Examiner: BRUMBACK, B.
Washington, D.C.
November 5, 2002
Docket No.: CLASSEN=1A

APPELLANT'S BRIEF UNDER 37 CFR §1.192

Honorable Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Sir:

In response to the final rejection mailed November 5, 2001, please enter the following appellant's brief.

The notice of appeal was filed May 6, 2002.

The small entity fee of $160.00 for this appeal brief is enclosed (credit card authorization form PTO-2038). Please charge any deficiency to deposit account 02-4035, and notify the undersigned.

1. FORMAL MATTERS

1.1. REAL PARTY IN INTEREST

The real party in interest is Classen Immunotherapies.

1.2. RELATED APPEALS AND INTERFERENCES

There are no pending related appeals and interferences. However, an Appellant's Brief was filed in this case on May 1, 2000. The Brief was entered, but on June 20, 2000, the Examiner reopened prosecution in order to "expand the rejections".

1.3. STATUS OF CLAIMS

Claims 5, 6, 8, 10, 11, 16, 27-30, 32, 41, 43, 44, 46, 49-52, 55-57, 59-68, 71-74, 77-78, 90-152, and 159 are pending (since entry of substitute amendment "A"). All other claims are

rejected.

We believe that the following rejections have been mooted by the entry of substitute amendment "A":

(1) prior art rejection of claim 19, now cancelled (November 5, 2001 rejection, page 13);

(2) enablement rejection of claims 149-152 vis-a-vis recitation of flavivirus antigens (November 5, 2001) rejection, page 10, fourth line from bottom to page 11, line 2);

(3) indefiniteness rejection of claims 149-152 vis-a-vis recitation of flavivirus antigens (Id., page 7, lines 1-4);

(4) indefiniteness rejection of claims 150-152 for being in improper Markush format (page 7, lines 5-7);

(5) indefiniteness rejection of claims 156 and 160 for dependency from cancelled claims (Id., page 7, lines 8-9);

(6) indefiniteness rejection of method claim 157 for dependency on kit claim 5 (Id., page 7, lines 10-11).

Substitute amendment "B", directed to claim 148, was intended to overcome the indefiniteness rejection stated in the last paragraph of page 6 of the November 5, 2001 office action.

Claims 5, 30, 56-57, 67, 71, 73, 144, 150 and 152 are subject to objections "for informalities in nomenclature". Claims 5, 30, 56, 71, 73, 144, 150 and 152 were amended by the June 21, 2002 substitute amendments "A" and "C" to overcome the objections. These amendments were entered, and we assume that the objections have been or will be withdrawn.

1.4. STATUS OF AMENDMENTS

The following amendments were filed after final rejection (status "entered"/"not entered" indicated in parentheses):

February 21, 2002 amendment after final rejection

(entry denied June 4, 2002).
Substitute Amendment "A" after final rejection filed June 21, 2002 (entered).
Substitute Amendment "B" after final rejection filed June 21, 2002 (not entered).
Substitute Amendment "C" after final rejection filed June 21, 2002 (entered).

Supplemental Amendment after final rejection filed Oct. 18, 2002 (awaiting decision)

2. SUMMARY OF THE INVENTION

Applicant has discovered that the timing of immunization with an immunogen, typically against an infectious disease, can affect the incidence or severity of a chronic immune-mediated disorder. Early immunization, typically prior to 42 days of age, appears to decrease the incidence or severity. See page 15, lines 2-10. Contrariwise, conventional pediatric immunization protocols, typically beginning at 6-8 weeks, actually can increase the probability that a mammal will develop a chronic immune-mediated disorder. See page 20, lines 11-15.

Applicant has already received a patent (5,728,385), issued on the parent application, for methods of reducing the incidence or severity of diabetes mellitis or systemic lupus erythrematosis by first administering an immunogen when the mammal is less than 42 days old (certain immunization schedules were excluded to avoid inherent anticipation).

Applicants have also received a patent (5,723,283) on a related application (a division of the corresponding PCT application) relating to methods of determining whether an immunization schedule affects the incidence or severity of a chronic immune-mediated disorder.

Finally, applicants have received a patent (6,420,139) on a continuation of the present case, with claims to methods of immunization comprising both screening various immunization

schedules for and immunizing humans according to a lower-risk schedule.

The instant claims are (1) additional therapeutic method claims, and (2) claims to kits for carrying out the desired immunization protocols.

3. ISSUES PRESENTED

I. Prior Art Issues

I/1. In the case of the kit claims, as rejected as anticipated by Madore, Dengrove, Halsey, John and Beneviste & Lagrange (OA §12), did the Examiner properly disregard the "labeling" limitation; more particularly, is there a "functional relationship" between the "labelling" ("printed matter") and the drug and its container ("substrate")?

II Description/New Matter Issue

II/1. Do the added limitations of claim 32 violate the "description" requirement?

II/2. Is there "description" for the label warning in claim 59?

II/3. Is there "description" for claims 40 and 145-148?

III. Enablement Issues

III/1. Does the present enablement rejection raise a utility issue, and, if so, must it satisfy the utility guidelines?

III/2. If so, is it sufficient under the Utility Guidelines to show that the assertion of utility/enablement is scientifically plausible?

III/3. What weight should be given to Applicant's epidemiological data as evidence of enablement?

III/4. What weight should be given to applicant's animal data as evidence of enablement in humans?

III/5. What weight should be given to evidence that immunization can increase the incidence of a chronic immune-

mediated disorder?

III/6. Is there a plausible mechanism of action as to how immunization could affect the risk of diabetes and, if so, what weight should be given to the existence of such a mechanism?

III/7. Has Applicant overcome the alleged prima facie case of Nonenablement vis-a-vis diabetes in humans?

III/8. Is the specification enabling for protection against chronic immune disorders other than diabetes, and especially for protection against autoimmune diseases, in particular, SLE?

III/9. Is the specification enabling for use of viral immunogens to elicit protection against diabetes?

III/10. Is the specification enabling for determining the effect of particular immunization schedules on the incidence or severity of chronic immune-mediated disorders?

IV. Definiteness Issues

IV/1. Is there antecedent basis for the limitations of claims 6, 11, 38 and 57?

IV/2. Is the language "total dosage greater than required for immunization against the infectious disease" (claim 146) definite?

IV/3. If so, may applicant properly recite "substantially greater" (claim 6)?

IV/4. Is "substantially reduces the incidence" (claims 27, 144, 145, 147) definite?

IV/5. Is the recitation of the "state of maturation" (claim 148) definite?

IV/6. Is the identification of immunogens by the disease caused by the source organism proper?

IV/7. Should the claims recite "herpes" or "herpes virus"?

IV/8. Is claim 148 indefinite because it recites a range without an explicit lower limit.

4. GROUPING OF CLAIMS

4.1. With respect to the rejection of the kit claims as anticipated by one or more references (issue I) the claims should be grouped as follows:

group I-A: claim 27 and all claims dependent thereon

group I-B: claim 59 and all claims dependent thereon

The basis for this grouping is the difference in the labeling limitation between claims 27 and 59.

With respect to the description issues (II/1 to II/3), these are addressed to specific claims, and no grouping is appropriate. II/1 applies to claim 32 and all claims directly or indirectly dependent thereon. II/2 applies to claim 59 and all claims directly or indirectly dependent thereon. II/3 applies to claim 40 and 145-148.

Issues III/1 through III/3 and III/5 to III/8 apply to all claims rejected for lack of enablement.

For issue III/4, the grouping is as follows:

A (claims reciting an animal model) (48, 170, 169)

B (claims reciting humans) (16, 43, 44, 46, 104, 105, 131-143, 168)

C (claims reciting equivalent maturation age)(102, 148)

D (all other claims).

For issue III/9, the grouping is as follows:

A: specific immunogens (5, 30, 55, 66-77, 91, 93-95, 121, 123, 124, 149-152)

B: bacterial immunogens (92)

C: immunogens more generally (all other claims)

This grouping is based on whether all immunogens are positively recited, or just subset. We have ignored negative limitations (all immunogens except X) for grouping purposes. However, if a claim were presented that exclude HSV, HCV, HIV and/or CMV, it would deserve to be separately grouped.

For issue III/10, the grouping is as follows

A: claim further limits immunization schedule, e.g., by time of first dose, number of doses, or interval between doses (8, 10, 11, 39, 40, 41, 50, 52, 57, 58, 146, 147, 159)

B: main claims, and dependent claims which do not further limit the immunization schedule (all other claims).

The definiteness rejections are directed to specific claims and hence no grouping is appropriate.

PRIOR ART ISSUES

5.1. (Issue I/1). In the case of the kit claims, as rejected as anticipated by Madore, Dengrove, Halsey, John and Benveniste & Lagrange (OA §12), did the Examiner properly disregard the "labeling" limitation; more particularly, is there a "functional relationship" between the "labelling" ("printed matter") and the drug and its container ("substrate").

The labeling is what the PTO calls "printed matter". Printed matter may constitute an element of a patentable claim and be given patentable weight, if there is a sufficient functional relationship between the printed matter and its substrate. See <u>In re Gulack</u>, 217 USPQ 401 (Fed. Cir. 1983); <u>In re Miller</u>, 164 USPQ 46 (CCPA 1969). Here, the printed matter explains how to use the substrate (the immunogen) to achieve the desired result (reduction in the incidence or severity of a chronic immune-mediated disorder).[1]

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[1] The "printed matter" doctrine is closely allied with the old "mental steps" and later "mathematical algorithm"

While, in a claim to a product, language of intended use is ignored, these kit claims require the presence of certain labeling. This is a tangible requirement, not a mere statement of intended use.

The Examiner maintains the rejection of the kit claims as anticipated by various references on the ground that there allegedly is not functional relationship between the printed matter and its substrate, as required by In re Gulack, 217 USPQ 401 (Fed. Cir. 1983) and In re Muller, 164 USPQ 46 (CCPA 1969).

What is a "functional relationship"? Presumably, it implies that without the printed matter, the substrate would be **less capable** of performing its function.

In the case of In re Miller, claim 10 read as follows:

> A measuring device comprising: a spoon for measuring ingredients; and volume measuring indicia defined in a normal volumetric unit on said spoon of a selected ratio to but indicating a volume different from the actual volume of ingredients being added to and measured in said spoon by said indicia, and a legend attached to said spoon specifying said ratio.

The court's opinion reproduces two apparatus of this type. In Fig. 2, we see a measuring cup with the legend "ONE HALF RECIPE", and various volumetric indicia. The line marked "2 CUPS" actually corresponds to a volume of one cup, so, if a full recipe called for "2 cups", by filling to the line in question, one would actually be adding the amount appropriate for a half recipe. In Fig. 3, we see a set of measuring spoons with a "½ recipe" tag. Here, the spoon marked "1

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doctrines, and, in this regard, it is interesting to note that an invention applying the rules and instructions for a game ("Cricket") to an otherwise old dart machine was held to be potentially patentable because the algorithm was not a mathematical one. See Arachnid Inc. v. Medalist Mktg. Corp., 18 USPQ2d 1941 (W.D. Wash. 1991). The claimed instructions for use do not define a mathematical algorithm.

teaspoon" has a true capacity of ½ teaspoon.

Were these indicia and legends to be removed, one would have cups and spoons worthless for accurate measurement. If just the legends were removed, one would have just a conventional looking (but inaccurate) measuring device or cup. The Court found that there was "a new and unobvious functional relationship between a measuring receptacle, volumetric indicia thereon indicating volume in a certain ratio to actual volume, and a legend indicating the ratio".

Similarly, in the instant kit claims, there is a new and unobvious relationship among "containers holding pharmaceutically acceptable doses of one or more immunogens" (which is like Miller's "receptacle") the "labeling" of the containers to indicate the identity and amount of each immunogen they contain (which is like Miller's "volumetric indicia")[2] and the "instructions" for use (which is like Miller's "legend").

The last of these points deserves particular emphasis. Miller's "legend" is an instruction for use. "One Half Recipe" is an instruction to the cook to use the cup or spoon se tin question when he or she wishes to prepare a "one half" recipe without recomputation of the required amount of each ingredient. Without the cook to interpret the legends and indicia, the cup and spoons do not perform any function. Their functionality resides in what they communicate to the cook. They do not help the receptacle hold more ingredients, or keep them fresher. They do not make the receptacle more watertight or airtight. Their relationship -- especially the legend's relationship -- to the receptacle is closely akin to the relationship exhibited by the printed matter in the instant kit claims to the immunogens of those claims.

In Gulack, the claim was to an educational device, which

[2] While this is not explicit in claims 27 and 29, it is an FDA requirement. The Supplemental Amendment, if entered, would make this explicit.

could take the form of a hat with a headband. Imprinted on the headband (the substrate) was a cyclic sequence of integers (the printed matter) obeying a particular mathematical rule. What was the functional relationship? According to the CCPA, the digits -- the printed matter -- were "related to the band in two ways: (1) the band supports the digits; and (2) there is an endless sequence of digits... exploit[ing] the endless nature of the band". In contrast, in the prior art Wittcoff reference, there was printed matter on the band, as in (1) above, but the data items were independent rather than arranged in a particular sequence.

Here, the labeling establishes a sequence, albeit temporal rather than spatial, for the use of the immunogens of the kit. Bear in mind that this relationship is between the printed matter and the immunogens, which are a part of the overall "substrate". In _Gulack_, the _distinguishing_ relationship was between one printed element and another printed element. Hence, the present case actually presents a stronger justification for the finding of a functional relationship than does _Gulack_.

While the immunogens are functional despite the labeling, that does not mean that a functional relationship is absent. Congress, in enacting the Food, Drug and Cosmetic Act (FDCA), recognized the existence of a functional relationship between a drug and its labeling. Thus, a new drug is not approved _per se_, rather it is approved for a particular indication (use). The new drug application includes "specimens of the labeling proposed to be used for such drug", see FDCA Sec. 505(b)1)(F). The FDA reviews the NDA and can refuse to approve if the testing was inadequate to show that "such drug is safe for use under the conditions prescribed, recommended or suggested in the proposed labeling thereof" (see FDCA Sec. 505(d)(1)) or the results "show that such drug is unsafe for use" or "do not show that such drug is safe for use" under "such conditions" (see FDCA Sec. 505(d)(2)). Moreover, approval may be refused

if "such labeling is false or misleading in any particular" (see FDCA Sec. 505(d)(7)).

Once a new drug has been approved, that approval may be withdrawn for the same reasons that approval could have been withheld in the first place. See FDCA Sec. 505(e).

Moreover, the FDCA draws a distinction, for <u>all</u> drugs, between adulteration and misbranding. If a drug contains a substance which is deletenous to health, it is adulterated. See FDCA Sec. 501. However, even a drug free of deletenous substances can be sanctioned if it is misbranded. A drug is misbranded if "its labeling is false and misleading in any particular", see FDCA Sec. 502(a). More significantly, it is misbranded "unless its labeling bears (1) adequate directions for use; and (2) such adequate warning against use in those pathological conditions or by children where its use may be dangerous to health, or against unsafe dosage or methods or duration of administration or application." See FDCA Sec. 502(f). A possible loophole is closed by FDCA Sec. 502(j), which says that a drug is "misbranded" if it is "dangerous to health when used in the dosage manner, or with the frequency or duration prescribed, recommended or suggested in the labeling thereof."

Prescription drugs dispensed by filling the prescription of a physician are exempt from Sec 505(f) and (j), cited above, but only if the drug bears a label presenting "the directions for use and cautionary statement, if any, contained in such prescription." FDCA Sec. 503(b)(2)

According to 21 CFR §201.57(e),

> Under this section heading, the labeling shall describe serious adverse reactions and <u>**potential safety hazards, limitation in use imposed by them,**</u> and steps that should be taken if they occur. <u>**The labeling shall be revised to include a warning as soon as there is reasonable evidence of an association of a serious hazard with a drug; a causal relationship need not have been proved**</u>.

Plainly, FDA realizes that some manufacturers and this consultants will argue their product has not been proven to cause a serious adverse event even though the data shows an association. FDA requires manufacturers to warn about a potential adverse event as soon as there is any reasonable evidence of an association. This is because it feels that the cost to the public of an unnecessary warning is much less than that of a delayed one.

While a physician may prescribe a drug for an off-label use without violating the FDCA, such prescription may be considered medical malpractice, and insurers may refuse to pay for such use.

We caution the Examiner against an overly restrictive definition of a "functional relationship", namely, that "without the printed indicia or numbers, the substrates lose their function." The case law does not justify that definition.

In Gulack the substrate was a headband. It remained functional as a headband, only its educational function would have been lost if the integer sequence were omitted. In Miller, the substrate was a measuring cup or spoon. It could still be used as a cup or spoon if the indicia were omitted. Thus, it is clear that neither case presented a substrate whose function was totally dependent on the indicia.

Here, it is true that the immunogen (if protective in its own right) could be used to protect against the corresponding infectious disease. However, without the claimed directions for use, the clinician would not know how to use it to limit the increased incidence or severity of the disorder attributable to late immunization.

In determining the functionality of an immunogen, it is appropriate to consider its side effects, not just its specific immunogen effect. If the side effects are detrimental, its functionality is reduced. If the side effects are beneficial, its functionality is enhanced.

The fact the immunogen has a residual level of functionality, absent the indicia, does not mean that there is no functional relationship between the immunogen and the indicia (labeling). If the latter increases the functionality of the immunogen, the necessary relationship exists and it is proper to give patentable weight to the labeling limitation.

An interpretation of "functional relationship" as meaning necessary for the functioning of the substrate is inconsistent with the alternative holding of the Federal Circuit in In re Lowry, 32 USPQ 2d 1031 (Fed. Cir. 1994). Lowry claimed memory for storing data which comprised a particular data structure (a pyramidal and hierarchical arrangement of "attribute data objects", ADOs), a data processing system comprising a database, a CPU, and memory means for holding the claimed data structure and methods of manipulating ADOs. The Examiner rejected the memory claim under ' 101, the system claims as obvious, and the method claims as anticipated. The Board reversed the ' 101 rejection, and upheld the prior art rejections. According to the Board, Lowry's data structures were analogous to "printed matter" and lacked a "functional relationship" to the substrate (the memory).

On appeal, the Federal Circuit held that because Lowry's data structures upon storage in memory, cause electromagnetic changes, there is a physical change, albeit invisible to the eye, and hence the data structures are not analogous to "printed matter".

However, it continued that even assuming that the analogy is valid, the Board erred in its reliance on Gulack. It pointed out that the ADOs enabled "more efficient data processing operations on stored data" in particular, that they "facilitate addition, deletion and modification of information stored in memory". The memory, of course, has a "function" even without Lowry's data structure. Lowry's merely structures "provided increased efficiency". However, that qualified as a "functional relationship": "In sum, the ADOs

perform a function, Gulack requires no more".

We also think it worth reiterating that if the labeling is given patentable weight (as we think proper as a matter of law), it is clear that the claims are not anticipated or rendered obvious by the reference. While it is certainly normal for an immunogen to be labeled with directions for use, to immunize against an infectious disease, and with warnings of side effects like acute toxicity, applicant was the first to teach that it should be labeled to direct its administration so as to limit the increased incidence and severity of a chronic immune mediated disorder (e.g. diabetes).

Consistent with this analysis, the PTO has allowed claims with "labeling" limitations.

Gerbe, USP 3,627,122, SYSTEM AND APPARATUS FOR THE ADMINISTRATION OF DRUGS (1971), claims an apparatus comprising compartmented trays, with "a patient and dose identification card" covering the bottom of each compartment, the card "having a folded portion...for holding said card in place". The claim also recites that each compartment has "a longitudinal pocket in one wall for a signal identification card".

Phykitt, USP 5,687,841, COMBINATION SHIPPING CONTAINER, MIXING AND DRINKING VESSEL (1997) claims the combination of analgesic medications and a package which serves both a shipping container and a mixing vessel. Claims 21-22 recite

> 21. The combination, according to claim 1, wherein said package further includes at least one of indications, directions, warnings, drug interaction precautions, active ingredients information and storage information disposed on an outer surface of one of said back portion and said front portion of said package.
>
> 22. The combination, according to claim 21, wherein said package includes each of said indications, said directions, said warnings, said drug interaction

> precautions, said active ingredients information and said storage information disposed on said outer portion of said back portion of said package.

Robertson, USP 5,752,723, PHARMACY LABEL AND PRESCRIPTION DRUG DISPENSING (1988) claims (18) "a labeled prescription drug package comprising...indicia comprising the name of a prescription drug, the dosage for proper administration of the drug, and the quantity of the drug to be provided in a package, imaged on said first label section".

See also Olney, USP 5,011,853 (claim 18= "a label which indicates that said pharmaceutical agent can be used for reducing the neurotoxicity of at least one cholinergic neurotoxin"); Kelly, USP 5,208,031 (claim 4= "the packaging material indicates that the sexual lubricant mixture... can reduce the risk of being infected by at least one type of sexually transmitted virus"); Sanders USP 4,820,635 (claim 1= "A kit ...comprising... instructions for performing the assay").

This is the first of several points in the brief in which we cite prior patents as evidence that a particular claim is acceptable. while we agree with the PTO that such evidence is not conclusive -- it certainly could not justify a legal position which was plainly contrary to the patent statute -- we cannot agree that is legally irrelevant. The courts have repeatedly found such evidence to be probative. Of course, the greater the number of patents cited, the more weight they carry. And the examiner is welcome to attempt to rebut the evidence of showing that a difference in the disclosure justified the difference in prosecution. However, the examiner cannot simply ignore the evidence.

The following cases illustrate the relevance of prior patents:

> Ex parte Brian, 118 USPQ 242, 245, (POBA 1958) (past practice of office in accepting definiteness of "fingerprint" claims);

> In re Chakrabary, 596 F.2d 952, 985-86 (CCPA 1979) (product claims reciting microorganisms previously treated as directed to statutory subject matter);
> Andrew Corp. v. Gabriel Electronics, Inc., 6 USPQ 2010, 2012 (Fed. Cir. 1988) (term "substantially" is "ubiquitous" in patent claims and therefore considered definite);
> In re Cortright, 49 USPQ2d 1464 (Fed. Cir. 1999) (Construction of "restore hair growth" for purpose of determining both §112 enablement and §101 utility; prior art references may be indicative of how a claim term will be interpreted by those of ordinary skill in the art);
> Vitronics Corp. v. Conceptronic Inc., 39 USPQ2d 1573, 1578-9 (Fed. Cir. 1996) (prior art used to demonstrate how a disputed term is used by those skilled in the art, and indeed is more objective and reliable than post-litigation expert opinion testimony);
> Pioneer Hi-Bred International v. J.E.M. Ag Supply Inc., 49 USPQ2d 1813, 1819 (N.D. Iowa 1998) (issuance of Boehm USP 2,048,056 in 1936 is evidence that "in those instances where inventors showed they could define a reproducible plant meeting the limits of §112, plant patents were issued under §101".)

The purpose of the patent system is to encourage innovation. The claims are a means of defining the invention in such a manner that it is reasonably clear what has been patented. It is one thing to reject a claim because it covers subject matter which is disclosed or suggested by the prior art, or which is not enabled. It is quite another to reject it on what amounts to stylistic grounds.

The PTO and the courts have recognized the propriety of once exotic claim formats-- "Jepson" claims, "Markush" claims, "product-by-process" claims, "fingerprint" claims, and claims

with "negative", "functional", or "alternative' limitations -- because they have realized that public policy demands that inventors not be hindered by hypertechnical claim drafting rules from fully protecting novel, nonobvious, and adequately disclosed inventions.

The instant "kit" claims are a case in point. Applicant has discovered that immunization can --depending on timing - either increase or decrease the incidence or severity of chronic immune-mediated disorders such as diabetes and SLE. A traditional product claim does not sufficiently protect applicant, as it cannot cover a prior art vaccine, even if that vaccine were used without consideration of its effect on a chronic immune-mediated disorder.

For a method claim to protect the invention, it must be crafted to avoid any instance in which the prior art use of a vaccine to immunize against an infectious disease might <u>inherently</u> (although inadvertently) have had the effect of also reducing the incidence or severity of a chronic immune-mediated disorder, as otherwise it could be held invalid on the ground of "inherent anticipation". Applicant has studied the literature, and has attempted to phrase the claim so as to avoid inherent anticipation, but simply cannot be sure that all such art has been avoided. An early immunization protocol might be set forth in an old or obscure journal anywhere in the world, or might have been used "publicly", without formal publication, in the United States. Indeed, the specification at page 31, lines 9-18 expressly recognizes the problem:

> The inventor appreciates that it is conceivable that a prior experimenter has, without recognition of its anti-chronic immune-mediated disorder activity, proposed or even practiced an immunization schedule which falls within the present disclosure, If, under the applicable law, such a proposal or practice would be deemed to anticipate or render obvious an invention here claimed, then it is within the inventor's contemplation to excise from the invention the specific embodiment in question, preserving to the maximum degree permitted by law the scope of protection originally sought.

A second problem with method claim protection is that it is geared to use of immunogens to <u>decrease</u> the incidence or severity of a chronic immune-mediated disorder. However, the Applicant has also enriched the art by teaching it to examine the chronic immune effects of conventional immunization. A vaccine manufacturer may find, after testing inspired by Applicant, that early immunization, while less likely to elicit this adverse effect, is also less effective against the infectious disease, and therefore continue to recommend, with appropriate warnings, late immunization. A "method of reducing the incidence or severity of a chronic immune-mediated disorder" claim would not reach this practice, even though the manufacturer would clearly have benefitted from Applicants's teachings.

A third problem is that the method claims are infringed by physicians. Applicant would prefer to assert direct infringement by the manufacturer. It is easier for Applicant to monitor vaccine labeling than to identify which doctors are following the claimed early immunization strategies.

A "kit" claim, like claims 27 and 59, solve these problems, without giving Applicant control of subject matter to which he is not entitled. Claim 27 and 59 are infringed only if the immunogen is distributed or sold with labeling either giving instructions which call upon the physician to practice the invention, or warnings indicating that the manufacturer has screened the immunogen as taught by Applicant.

Claims 27 and 59 could not be inherently anticipated by the naive use of the immunogen in an early immunization schedule, since such use, by definition, would make no reference to the effect of the immunogen on the incidence or severity of a chronic immune-mediated disorder.

Thus, we have explained why the functionality of the immunogens here should be deemed to be affected by the labelling, per <u>Miller</u> and <u>Gulack</u>. As for <u>In re Giolito</u>, 188

USPQ 645 (1976) (1976), cited by the Examiner in the office action of November 5, 2001, page 13, this hardly overrules the numerous post-1976 cases which have given weight to prior patents, see above.

6. DESCRIPTION ISSUES

OA §7 says that the rejection of claims 5, 6, 8, 10-11, 16, 30, 32, 38, 49, 55-57, 59-65, 72 and 74-101 under 35 U.S.C. 112, first paragraph, for "new matter" is maintained for reasons of record. Additionally OA §8 rejects claims 40 and 145-148 for lack of description.

Technically speaking, if new matter is allegedly added to a claim, the rejection is properly termed a rejection for "lack of written description", not a rejection for "new matter". See MPEP §2163.06(I).

Turning first to OA §7, it appears that the issues are:

(1) the propriety of the added limitations to claim 32 (Issue II/1) (see February 21, 2002 office action, page 6, para. 1)

(2) the propriety of the label warning in claim 59 (Issue II/2) (see February 21, 2001 office action, page 6, para. 2).

We believe that the other claims rejected in OA §7 for "new matter" are rejected solely because of their dependency on claims 32 or 59, and hence they need not be separately addressed.

In OA §8, the Examiner questions the following language of claims 40 and 145-148:

40 ("at least one")
145 ("four different dates")
146 (total dosage in first 112 days)
147 (not pertussis, and at least three doses)
148 ("the first dose...mouse or a rat")

6.1. Issue II/1: Do Added limitations of claim 32 violate the "description" requirement?

Claim 32 was amended to introduce the following limitations:

(1) if only one immunogen is administered, it is other than BCG;

(2) if the one immunogen is whole cell pertussis, the schedule is one other than a schedule of three doses at one week intervals, all given in the first month; and

(3) if all the immunogens administered are selected from a list of 10 immunogens, either

(a) one or more immunogens are administered on at least three different dates prior to 42 days after birth, or (b) one or more immunogens are administered on at least three different dates, and the maximum interval between administrations is about two weeks, or less.

Limitations (1) and (3) were copied from claim 1 of Classen, USP 5,728,385, which issued on the parent application, except that claim 32 refers to "one or more immunogens" instead of just "immunogens" to make it clear that a single immunogen could be administered. Note that the immunogens administered on different dates could be the same or different.

The Examiner says that because this case is a CIP of the prior case, and does not incorporate the prior case by reference, he cannot assume that just because there was descriptive basis in the parent case (as implied by the issuance of a patent) that there is descriptive basis here.

With regard to the "other than BCG" limitation in (1), the limitation appears to be intended to excise prior art like that of Grange and Stanford (1990) cited at page 6, lines 11-14, and Harada (1990), cited at page 9, line 16 to page 10,

line 4, and hence "described" by page 31, lines 9-18:

> The inventor appreciates that it is conceivable that a prior experiment has, without recognition of its anti-chronic immune-mediated disorder activity, proposed or even practiced an immunization schedule which falls within the present disclosure. If, under the applicable law, such a proposal or practice would be deemed to anticipate or render obvious an invention here claimed, then it is within the inventor's contemplation to excise from the invention the specific embodiment in question, preserving to the maximum degree permitted by law the scope of protection originally sought.

Moreover, original PCT[3] claim 1 (which automatically has "description") recited "said one or more immunogens... optionally including at least one immunogen other than BCG". See also original PCT claims 5 ("other than BCG,... yellow fever", total of 21 immunogens listed) and claim 7 ("other than BCG...also...other than smallpox").

Turning to limitation 3(a), original PCT claim 13 provides we have already explained the basis for at least three dosings prior to 42 days after birth ("where within the first 42 days after birth, at least one immunogen is administered in at least two, more preferably at least three, distinct dosings"). With regard to 3(b), the basis for at least three dosings (not necessarily all prior to age 42 days) is in original PCT claim 9 ("at least one immunogen is administered in at least two, more preferably at least three, distinct dosings") and for a maximum interval of about two weeks, in original PCT claim 11 ("wherein, during the first 175 days from birth the longest interval between two

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[3] This application is the national stage of PCT/US94/08825. This PCT application originally presented 24 claims. In IPE, original claims 1, 3, 4, 7, 12, 13, 18, 23 and 24 were deleted, and 25-30 were added. On national stage entry, a preliminary amendment cancelled 20 and 22 and added 31-33.

successive dosings of at least one immunogen is... preferably less than or about 14 days"). The ten immunogens in question are BCG (from original claim 1) plus those listed in original PCT claim 4 (with the possibly inadvertent exception of hepatitis A). These are the ten pediatric immunogens listed on page 35, lines 24-26, and hence the ones for which the risk of inherent anticipation was greatest.

Limitation (2) was introduced to avoid any possibility of inherent anticipation by Adams (1947) (of record)[4], as cited in Table 5 of Halsey (of record). Excission of a prior art species from a generic claim is proper, see In re Johnson, 194 USPQ 187 (CCPA 1977) and indeed was contemplated as a possibility, see page 31, lines 9-18, previously quoted. The Halsey article is cited in the specification (p. 109) and incorporated by reference, as are all articles (including Adams) cited by Halsey. See pp. 99-100. Hence, there is no violation of the "description" requirement.

We would add that there is specific support for giving at least three doses (original PCT claims 9, 12 and 13), for one week intervals (original PCT claim 10), and for first administration at 7 days old (original PCT claim 8)[5].

The Advisory Action of September 29, 1999, at page 8 raised the issue of whether Applicant could rely on the language of the original PCT claims. It is well established that the original claims of a U.S. application are a part of the original description. See MPEP § 2163.03(I), citing In re Koller, 204 USPQ 702 (CCPA 1980).

The question is whether, when a PCT application is filed which designates the U.S., and the PCT claims are amended during IPE, whether the "original" claims for purpose of 35 USC § 112 include the PCT claims as filed.

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[4] Adams immunized with "phase I superconcentrate vaccine", with a total dose of "100,000,000,000 organisms".

[5] IPE claim 9 also supported "at least one immunogen other than pertussis".

We have two independent bases for urging that they are, at least in this case.

First, 35 USC § 363 clearly states "an international application designating the United States shall have the effect, from its international filing date under article 11 of the treaty, of a national application for patent regularly filed in the Patent and Trademark Office except as otherwise provided in section 102(e) of this title." Clearly § 102(e) has nothing to do with the "description" requirement, which is based on § 112. So the international application as filed, with claims 1, 7, 12 and 13, has the same effect as a U.S. application filed that day.

Secondly, the Examiner's attention is respectfully directed to section 16 of the transmittal letter, item 4

> "A courtesy copy of the International Preliminary Examination Report with annexes. Note: Please use the claims as they appear in the IPER annexes as the claims in this case. Claims indicated as "deleted" in the annex should be deemed presented on filing but cancelled herewith by preliminary amendment, to avoid renumbering."

Hence, the PTO was instructed to treat original PCT claims 1, 7, 12 and 13 as if they had been presented at the time of national stage entry, and then, a moment later, cancelled. The original claims of a U.S. application are part of the description even if they are subsequently cancelled.

6.2. Issue II/2

Is there "description" for the label warning in claim 59?

With respect to the label warning of claim 59, the Examiner says that there is no reference to warning labels or instructions per se at pp. 51-52, p. 7, l. 11-14, or p. 54, l. 14-21.

Page 7, lines 11-14 states

> The lack of concern over the ability of vaccines to induce a chronic immune mediated disorder (e.g., but not limited to, diabetes) is further evidenced by the lack of warnings on package inserts and labels of such products about such diseases.

Thus, the specification explicitly criticizes the prior art vaccine labeling for failing to warn about the ability of vaccines to induce a chronic immune-mediated disorder.

Since the specification warns that vaccines <u>can</u> induce a CIMD, it follows that warnings should be provided.

Furthermore, at page 54, lines 14-21, the specification states

> Alternatively, a screening trial may be designed to determine if an immunization schedule, such as a standard schedule known in the art, will <u>induce and/or enhance</u> the incidence and/or severity of at least one chronic immune mediated disorder. In the latter case, it may be especially useful in screening production lots of approved vaccines for the hitherto unrecognized safety problem of inducing or exacerbating a chronic immune-mediated disorder.

If such induction or enhancement is detected, and the vaccine is still sold with labeling calling for the schedule in question, the law would require a warning.[6] FDA practice requires one to place warnings on a package insert if there is a potential adverse event, and this is well known to those

[6] The Code of Federal Regulations is cited at page 42, line, and page 46, lines 24-25, and hence incorporated by reference at page 99, lines 22 to page 100, line 2. The CFR cites the Federal Food, Drug and Cosmetic Act, and hence the latter is also incorporated by reference, see page 99, line 27 to page 100, line 2. We can, of course, amend the specification to explicitly insert relevant portions of the CFR or FDCA into the specification, without adding "new matter".

skilled in the art. See, e.g., 21 CFR 201.57(e).

As the Examiner is well aware, it is not necessary that the exact language of the claim appear in the specification in order to satisfy the "description" requirement. In re Lukach, 169 USPQ 795, 796 (CCPA 1971); In re Edwards, 196 USPQ 465 (CCPA 1978); In re Smythe, 178 USPQ 279 (CCPA 1973) ("fluid" described by "gas"). The specification is directed to a person skilled in the art, who therefore would be aware of FDA labeling requirements.

Page 54, lines 14-21 contemplates screening standard immunization schedules for the ability to "induce and/or enhance the incidence and/or severity of at least one chronic immune-mediated disorder". Suppose that such a screen were positive. If so, then under the food and drug laws, it would be necessary to mention this adverse effect in the labeling. See 21 CFR §201.57(e), previously quoted.

Page 51, lines 26-28 teaches that the kits will be "in forms suitable for pharmacological administration". Arguably, if an immunogen were screened per page 54, and found to cause diabetes under the contemplated immunization schedule, the FDA would require a warning on the labeling and a kit without such labeling would not be "suitable for pharmaceutical administration".

Original claim 2, which is part of the "description", stated:

> The method of claim 1 where said mammal is not immunized with an immunogen in such amounts and at such times as would substantially induce an immune-mediated disorder.

Since manufacturers of vaccines have no control of how the vaccines are used by physician-purchasers, a label warning is plainly a contemplated means of accomplishing what is suggested by original claim 2.

6.3. Issue II/3

Is there "description" for claims 40 and 145-148?

We set forth the basis for claims 144-148 at pp. 10-11 of the August 17 amendment. The Examiner has specifically questioned some of the language of claims 40 and 145-148, which we defend as follows.

Claim 40

This claim recites that "at least one immunogen is given in two or more dosings such that the shortest interval between two successive dosings thereof is **at least one and** less than 28 days". [emphasis added]

This limitation differs from the most general limitation of original PCT claim 10 solely in that the boldfaced language was inserted because the examiner criticized the prior version of the claim as indefinite because it failed to recite a lower limit.

According to page 26, lines 8-11, "for the purpose of the appended claims, the administration of two different immunogens, or of two packets of the same immunogen, within a period of less than 24 hours, is considered a single dosing". It follows that the interval between dosings as thus defined cannot be less than 24 hours (1 day). We believe that page 26, lines 8-11 is in itself sufficient to justify a lower limit of 1 day.

In Example 2 and 5 the shortest interval was two days (between dosings on days 1 and 3), see page 83, lines 16-17, and page 88, line 10.

In Example 4, the shortest interval was three days (between dosings on days 1 and 4), see page 87, line s9-10.

However, if "two packets of the same immunogen" can be given "within a period of less than 24 hours", as well as at an interval of two or three days, it seems clearly contemplated that they can be given at an interval of one day.

Claim 145

This claim requires that "at least one immunogen is administered on at least four different dates prior to 42 days after birth".

Original PCT claim 12 contemplated that there could be "at least four...dosings" within "the first 112 days after birth". Original PCT claim 13 contemplated that there could be at least three dosings within the first 42 days".

In Schedule 1 on page 107, 10 different immunogens are given <u>four</u> times within the first 42 days, specifically, in weeks 0, 2, 4 and 6 (week 6 = 42 days). Schedule 4 is similar, except that only five immunogens are so administered.

Claim 146

The final clause of this claim recites "wherein for at least one such immunogen, the total dosage during the first 112 days after birth is greater than that required for immunization against the infectious disease with which it is associated".

This differs from original PCT claim 6 only in that the latter had recited "substantially greater". Claim 146 omitted "substantially" to avoid indefiniteness issues (see issue ___, below). However, this cannot give rust to a "description" problem as, it is "substantially greater" than B, it necessarily is "greater" than B.

Claim 147

This claim has been criticized because it recites "wherein at least one immunogen so administered is one other than pertussis, and a plurality of doses of that immunogen are administered:

The "pertussis" exclusion is based on original PCT claims 4 and 5. It is also noted that in example 2, one of the groups received two immunogens (tetanus and diphtheria) in addition to pertussis, and another received just diphtheria and tetanus, and not pertussis at all.

The "three dose" limitation is based on page 26, line 4.

Claim 148

Finally, with regard to claim 148, the Examiner criticizes the third paragraph:

> where, if only one immunogen is administered according to said immunization schedule, that immunogen is one other than BCG, and, if said one immunogen is whole cell pertussis, the schedule is one other than a schedule of three doses at one week intervals, all given in the first month,

The issue of relative maturation rates is addressed in great detail at page 27, line 15 to page 29, line 19. Perhaps the most relevant passage is the one at page 29, lines 13-19:

> The present invention therefore can include administration of the immunogens to humans when said humans' immune systems are in a state of maturation and responsiveness comparable to that of mice or rats that the times indicated above, in such circumstances as it would be less effective to administer those immunogens to humans at the same chronological ages as they were administered to mice or rats.

This plainly justifies the questioned limitation of claim 148.

7. ENABLEMENT ISSUES

OA §9 rejects method claims 5, 6, 8, 10, 11, 15, 16, 19, 27-30, 32-41, 43, 44, 46, 49-52, 55-57, 58-101, 103, 106 and 128-143 as "not enabled" for "reasons of record".

OA §10 likewise rejects newer claims 144-152, 156, 157, 160 for lack of enablement.

7.1. Relationship to Prior Patents

At the outset, we would like to point out that the claims at issue are essentially parallel in scope to those granted in

USP 5,728,385 and USP 5,728,283. These patents allowed claims which generically recited administering "immunogens" to "mammals" to decrease the incidence of a "chronic immune-mediated disorder". Moreover, USP 6,420,139 allowed claims which generically recited administering "immunogens" to humans to reduce the risk of diabetes. Those patents are presumptively valid under 35 USC §282. Moreover, the actions of the prior examiner in those cases is entitled to full faith and credit.

7.2. <u>Summary of Office Actions</u>

Applicant filed a brief on May 1, 2000. However, on June 20, 2000, the Examiner reopened prosecution to "expand the rejections". Enablement was addressed in OA §7.

The Examiner argued that Halsey, et al., PIDJ 18: 217-22 (1999)("PIDJ") teaches that available data are inconclusive with regard to a protective effect of vaccines against development of diabetes in humans. She then went on the discuss three studies reported in PIDJ.

Based on the results of these studies, she argued that applicant's animal model data and human epidemiological data (called "ecologic studies") is inconclusive as to efficacy in humans.

The Examiner also cited Boumpas to the effect that the underlying pathogenesis of SLE is unknown, complex, and different from patient to patient.

The Examiner also questioned how the immunization protocols for the diabetes models are to be adapted for other autoimmune diseases.

We assume that any issue raised by a prior rejection which was not reiterated in the June 20, 2000 office action was <u>impliedly withdrawn</u>, unless it was explicitly raised in a later action.

In the December 9, 2000 response, Applicant pointed out that the PIDJ article was produced by the Institute for Vaccine Safety IVS) and argued (with supporting exhibits) that

the IVS had a vested interest in allaying public concerns that immunization could increase the incidence of diabetes. Response, pp. 16-17.

In 2001, the Examiner acknowledged some of the bias evidence (Exs. A2 and E2, but not M1 and M1B), but concluded that the evidence was not directly relevant.

The Examiner also cited additional references allegedly supportive of the conclusions set forth in PIDJ: DeStafano (DU), EURODIAB (EB), Graves (EF), Heijbel (EI), Hiltunen (EL), Jefferson (EG), Karvonen (EV), Bedford (HD), Petousis-Harris (HE), Dahlquist (HK), Jefferson (HP), Elliott (IJ) and Anonymous (IN). Some were already cited by PIDJ.

The December 9, 2000 response had pointed out that the standard applied was inconsistent with the utility guidelines, but the examiner held this was not germane as the rejection was for enablement.

The Examiner reiterated her position that "epidemiological data alone does not establish a causal relationship", without commenting directly on the fact that it did not stand <u>alone</u> (see Classen's mouse and rat data).

The Examiner questioned the extrapolation of animal data to humans "because of the criticality of the age of administration and the differences in maturation rates between rodents and humans", citing Elliot (IJ).

Finally, the Examiner discounted the reports that vaccines may <u>cause</u> chronic immune-mediated disorders (exhibits 1E, 5G, 1A, 5H, 5E and Classen references) because the claims were to reducing CIMD, not the converse.

These points were responded to on August 17, 2001. The November 15, 2001 final office action mostly reiterated old arguments, without amplification. However, it alleged on page 9 that the Classen & Classen reference taught a correlation between <u>increased</u> risk and immunization, and hence supposedly taught <u>away</u> from the claims.

<u>7.3. Evidence of Enablement/Operability</u>

Before responding to the rejections in detail, we would like to lay out applicant's primary evidence of enablement/operability.

7.3.1. ANIMAL DATA

The present specification presents five experimental examples, which are summarized below.

Example 1

Shows reduction in incidence of diabetes in NOD mice receiving (a) anthrax or (b) plague, on days 8, 15 and 29. The anthrax was more potent.

Example 2

Shows further reduction in incidence of diabetes in NOD mice receiving anthrax on days 1, 3, and 10, and weeks 4, 6, 8, 10, 12 and 14. Still further reductions obtained by combining the anthrax with tetanus and diphtheria, and even more with pertussis also provided.

Also shows that first immunization of NOD mice with DTP at 8 weeks leads to <u>higher</u> incidence of diabetes.

Example 3

Mice were injected with cyclosporine to make them prove to developing autoimmunity, and then were immunized with (a) anthrax + diphtheria + tetanus (ADT) at days 10, 17, 31 and 45 and (b) anthrax + DTP at days 6-8, 14-16 and 27-29 (3 admin.). Both treatment groups exhibited decreased incidence of anti-(gastric antigen) autoantibodies, with the effect being greater for group (b).

Example 4

BB rats (another diabetes model) were immunized with anthrax + DTP at days 1, 4, 11, 25, 39, 53, 61, 75, 89 and 103. They showed decreased incidence of diabetes relative to

control rats.

Example 5

MRL/MPJ-lpr mice were used as a model of SLE. The mice were injected with anthrax + acellular DTP at days 1, 3, and 10, and weeks 4, 6, 8, 10, 12 and 14. The incidence of glomerulonephritis (a symptom of SLE) was reduced by this immunization.

7.3.2. HUMAN EPIDEMIOLOGICAL DATA

Example 101 and Tables I-IV

Additionally, epidemiological data is presented in Example 101 and Tables I-IV. Table I compares different countries, with different immunization plans, for the same time period (roughly 1980-1990), while the other tables look at the effect of temporal changes in immunization schemes in a single country. Table I examines the effect of pertussis and BCG immunizations in various countries; Table II shows changes in the incidence of diabetes in Finland, explained at pages 93-95 as attributable to use of Hemophilus influenza and MMR vaccines. Table III focuses on Allegheny County, Pennsylvania, and the discussion at pages 95-97 correlates changes with usage of Hemophilus influenza, pertussis and mumps vaccines. Finally, Table IV is said at pages 97-99 to evidence a connection between smallpox immunizations and diabetes. The first immunization was given earlier at the time of a smallpox epidemic.

Post-Filing Evidence

Earlier in prosecution, we made reference to Classen and Classen, Infect. Dis., 6:449-454 (1997); and Classen, J.B., and Classen, D.C. "Immunization in the first month of life may explain decline in incidence of IDDM in the Netherlans" Autoimmunity 31:43-45 (1999). See also the declaration of Dr. Classen executed July 8, 1994 and filed the same day in Serial

No. 08/104,529, now USP 5,728,385, and the Classen declaration filed September 7, 1999, and addressing hepatitis B virus immunization.

On Oct. 18, 2002, we filed a new declaration from Dr. Classen, enclosing various supporting exhibits. These exhibits are summarized in Table 1 below, which is copied from that Declaration. Some of these exhibits were previously submitted, and others were prompted by the final rejection (see exhibit list attached to declaration for details).

7.4. Analysis of Enablement Issues

Issue III/1. Does the present enablement rejection substantively raise a "utility" issue, and, if so, must it satisfy the utility guidelines?

According to MPEP §2164.07, there are actually two kinds of rejections under 35 USC §112, first para.

The first is imposed when the examiner asserts that there is a lack of a credible basis for believing the claimed utility of a method (or any disclosed utility of a claimed product). In this case a dual rejection is made under §112 ¶1 and under §101, and the rejection must provide the factual showing demanded by the Utility Guidelines, MPEP §2197-2107.03.

The other rejection is one which concedes that the disclosed compounds could be used for the disclosed utility, but asserts that undue experimentation would be necessary to identify the specific conditions necessary for success. In this case a rejection is made only under §112 ¶1, and the standard of MPEP §2164.01-.06 is applied.

If a rejection substantively raises a "utility" issue, then the Utility Guidelines should be applied, even if the rejection avoids the word "utility" and speaks of "undue experimentation".

Conceding that the invention works with specific immunogens to control diabetes in mice does not dissipate the

larger "utility" issue.

As we first pointed out in §4.1 of the August 17, 2001 response, with regard to enablement vs. utility (OA page 8, lines 6-9), in our view, when the Examiner questions the believability of a utility, she is making a utility rejection, while when the Examiner questions the quality of the written disclosure of a believable utility, she is making an enablement rejection. See MPEP 2164.07. Here, the examiner questions extrapolation of animal data (see Issue III/4 below), which is a typical utility issue, see MPEP 2107.02 (III). In other words, this is a utility rejection in enablement rejection clothing, and the utility guidelines should apply. Hence, procedurally, these utility issues should be raised in a combined 101/112 ¶1 rejection, and any pure enablement issues in a separate 112 ¶1 rejection, see MPEP 2164.07.

Issue III/2. If so, is it sufficient, under the Utility Guidelines, to show that the assertion of utility/enablement is scientifically plausible?

The PIDJ article says, "no vaccines have been shown to increase the risk of type 1 diabetes in humans". Even if this statement were taken at face value, it does not address the utility standard under the patent law. The question is where the asserted utility is "credible", not whether it has been proven.

When draft utility Guidelines were introduced in 1994, Commissioner Lehman commented

> The guidelines emphasize that any credible statement of utility consistent with the scope of the claimed invention that is made by an applicant will satisfy §101. In other words, if an applicant presents a scientifically plausible use for the claimed invention, it will be sufficient to satisfy the utility requirement. [emphasis added]

Consistently, the current March 1, 2000 Training

Materials comment

> An assertion is credible unless (A) the logic underlying the assertion is seriously flawed, or (B) the facts upon which the assertion is based are inconsistent with the logic underlying the assertion. Credibility as used in this context refers to the reliability of the statement based on the logic and facts that are offered by the applicant to support the assertion of utility.

In reviewing the evidence relied on by the Examiner, or it is important to keep in mind that Dr. Classen teaches that the incidence of diabetes is affected by the timing of immunization: beginning immunization early reduces incidence, while starting it late increases incidence. Thus, a study that merely looks at the effect of giving a particular immunogen on diabetes, and ignores the timing of the immunization, may overlook a real connection, especially if there was substantial variation in timing from subject to subject within the immunized group.

Issue III/3. What weight should be given to Applicant's Epidemiological Data as Evidence of Enablement?

Within issue III/3, certain "subissues" can be recognized:

(1) is epidemiological data relevant even though it cannot prove a causal relationship?

(2) did Applicant properly analyze and interpret his epidemiological data?

(3) are there epidemiological studies by others which reach a different conclusion, and, if so, were they properly conducted?

(4) are there epidemiological studies by others which support applicant and, if so, were they properly conducted?

(5) to the extent that properly conducted studies

reach contradictory conclusions, is there any way they can be harmonized?

(6) on balance, do the various epidemiological studies by applicant and others, duly weighted to account for the quality and relevance of the study, support the scientific plausibility of Applicant's claimed approach to reducing the risk of diabetes?

III/3(1) is epidemiological data relevant even though it cannot prove a causal relationship?

The Examiner says that "epidemiological data alone does not establish a causal relationship". That is true, but it can render a proposed utility <u>believable</u>.

The scientific community often must rely on epidemiological data to establish causation. It is unethical to perform a clinical trial with a suspected toxic substance in order to "prove" the substance is toxic. Therefore epidemiology data alone is suffice to establish casual relationship for practical purposes. For example no one has ever done a prospective study to establish that cigarettes cause disease. The establishment of a casual relationship between cigarettes and disease is based on epidemiology data. The same goes with almost all toxins, for example asbestos, carcinogenic chemicals, radiation, toxic chemicals.

The Examiner's objections apply to any epidemiological study, and <u>In re Irons</u>, 144 USPQ 351 (CCPA 1965) held that use of "historical controls" is acceptable. Moreover, the epidemiological data does not stand alone.

III/3(2) Did applicant properly analyze and interpret his epidemiological data?

The examiner did not specifically criticize the design of any of Dr. Classen's epidemiological studies, or the statistical methods by which he analyzed their data. Instead,

the Examiner relied on the criticism appearing in certain cited references.

The epidemiological studies by Classen or others which have reached conclusions favorable to applicant are set forth in Table 1 below. For each study, the left column summarizes the study parameters, data and conclusions, while the right column describes any criticism of that study which has appeared in the art of record, and Applicant's response thereto.

This review compels the conclusion that Applicant's epidemiological studies were properly conducted and constitute substantial evidence of enablement and utility.

Table 1: Epidemiological Studies with Conclusions favorable to Applicant

(Left col. description of study and its findings; right col. description of any critiques, and applicant's rebuttal.)

1. Pertussis/BCG/Hib, Western Europe Classen EX. 101 and Table I; see also Classen and Classen, "The Timing of Pediatric Immunization and the Risk of Insulin-Dependent Diabetes Mellitus", Infectious Diseases in Clinical Practice (IDCP), 6: 449-54 (1997), Table 2	
incidence of diabetes correlated to immunization schedule for Western european countries in period 1980-1990, i.e. (1) no pertussis, no BCG (16.6), (2) pertussis, BCG before two months (7.4), (3) pertussis, but no BCG (10.92), (4) pertussis, BCG vaccination at school age (19.02), and (5) pertussis, BCG, Hib vaccination at 3 months and at school age (42.9). **There were highly significant differences in incidence between several groups**	The examiner has not pointed out any methodological flaws in this analysis, or cited any papers which do so. The examiner did cite PIDJ, which alluded to intercountry analyses by Moulton and LaPorte (see below)

2. Hib/MMR, Finland, 1970-1989 Classen pp. 93-95; see also Classen and Classen, IDCP (1997), table 3.	
changes in the incidence of diabetes were correlated to changes in immunization schedule in Finland, namely, (1) a large clinical trial (130,000) started Nov. 1974, of Hib or meningococcal polysaccharide vaccines, (2) increase in the antigenicity of the pertussis vaccine in 1976, (3) addition of measles, mumps and rubella in 1982, and (4) another large Hib vaccine clinical trial (114,000) initiated in Jan. 1986, and (5) addition of Hib to standard schedule in Jan. 1988. Data were stratified into periods 1970-76, 1977-79, 1980-82, and 1987-89, and into 0-4, 5-9 and 10-14 years old age groups. Large percent increases in incidence were seen in the two younger age groups in 1977-79, and in 1987-89. **The differences in incidence from one period to the next were significant, in some cases highly so.**	The examiner has not pointed out any methodological flaws in this analysis, or cited any papers which do so. The examiner did cite PIDJ and other papers which address Classen's analysis of the effect of a specific Hib trial in Finland, see below.

3. Pertussis/mumps/Hib; Allegheny County, Pennsylvania, 1965-1989 Classen pp. 95-97	
Changes in the incidence of type I diabetes were correlated with the changes in the immunization schedule in this county: (1) 1975 Pennsylvania legislation implying that pertussis immunization was not necessary; (2) increased pertussis immunization following a 1982 epidemic; (3) state law requiring mumps vaccination (1983); and (4) addition of Hib vaccine (polysaccharide in 1985, conjugated vaccine in 1987). Data were stratified into periods 1965-69, 1970-74, 1975-79, 1980-84, and 1985-89. The incidence decreased in 1975-79 (-59%) and increased in 1980-84 (276%) and 1985-9 (63%). These three changes were highly significant.	The examiner has not pointed out any methodological flaws in this analysis, or cited any papers which do so. No papers providing contradictory new data for the county have been cited.

4. Smallpox: Netherlands Classen p. 97, L8-14; P98, L12-P99,L4; Table IV; Classen and Classen, "Immunization in the First Month of Life may Explain Decline in Incidence of IDDM in The Netherlands," Autoimmunity, 31: 43-5 (1999)(Ex. 5A)	
The practice in the Netherlands during 1960s was to immunize for smallpox at 2 months of age during non-epidemic conditions and earlier (perhaps at birth) during epidemics. The Classen appl. reports that cohorts born during smallpox epidemics had a lower incidence of type I diabetes then those born at other times. The implication is that **early** administration of the smallpox immunogen **reduced** the incidence of diabetes. **Table IV** Correlates cumulative rate of incidence of type I diabetes in Dutch[7] military recruits with the number of smallpox cases in Europe in the year of birth of the recruit, for the period 1960-1970. There was a statistically significant decline in 1962, which was the year of a smallpox epidemic. There was also a decline in another epidemic year, 1966, but this decline was not statistically significant. Classen concludes that the declines were attributable to changes in immunization practice in response to the epidemic.	The examiner has not commented on this observation. The examiner generally cites PIDJ, which in turn cites Blom (see Table 2A below). The Blom study considered smallpox immunization in <u>Sweden</u>, observing a RR of 1.07 (conf. 0.77-1.49) . While the noted effect (while consistent in direction and magnitude with Classen's studies), was not statistically significant, at the time of the Blom study (cases reported in 1985-86), smallpox had long been excluded from the general Swedish vaccination program. The power of the study to detect the effect of smallpox immunization was therefore low.

[7] Mislabeled as "Danish" military recruits in Table IV and on page 97 of the specification. We are filing a supplemental amendment to correct this. The error is evident from inspection of the primary source, Drykoningen, et al., "The incidence of male childhood type 1 (insulin-dependent) diabetes mellitus is rising rapidly in the Netherlands, Diabetologia, 35: 139-42 (1992)(copy enclosed) cited at the bottom of Table IV. The number of diabetes cases is plainly taken from an article on the incidence of diabetes in **Dutch** military recruits.

<table>
<tr><th colspan="2">5. BCG: Sweden
Classen and Classen, IDCP (1997): Table 1</th></tr>
<tr><td>BCG was routinely administered at birth to all newborns in Sweden until April 1975. The cumulative incidence of diabetes in the 1973-77 birth cohorts was studied. The difference between (1976-77) and (1973-74) was 32.2, with a one-tail P value of .0363. The difference between 1974 and 1976 was 48.64, with a one tail P value of .0028. Classen used a one-tailed test because, based on his earlier epidemiological studies, he expected BCG at birth to decrease the risk of diabetes. However, the 1974/1976 comparison would have resulted in a finding of high significance even with a two-tailed test (P then .0056).

Thus, early immunization with BCG was associated with a lower incidence of diabetes in later life, as compared to unvaccinated controls.</td>
<td>The examiner has not commented on this observation. The examiner cites PIDJ, which in turn cites Blom.

Blom (1991) (see below) table 3 reported an odds ratio of 1.04 (conf 0.77-1.4) for tuberculosis immunization. Blom looked at 0-14 yr old diabetes cases reported in 1985-86, but BCG was mandatory only til 1975. So Blom's cases were either children who developed the diabetes relatively late, or younger children considered to be high risk for tuberculosis who received voluntary BCG immunization.

In applicant's study, the cohort sizes were 95,000-109,000. Blom's evaluation of the effect of tuberculosis vaccination was certainly based on a smaller number of cases. Hence, it is not surprising that he had a broad confidence interval.</td></tr>
</table>

6. Hepatitis B, New Zealand Classen and Classen, IDCP (1997), Table 4; Id., "Diabetes Epidemic Follows Hepatitis B Immunization program," New Zealand Med. J., 109: 195 (1996).	
Classen presents data on the incidence of type I diabetes in Christchurch, New Zealand, 1982-1991. A massive hepatitis B immunization program was introduced in 1988, with first immunization generally starting after 6 weeks from birth. Initially, children under 5 were immunized, but the program was externed over the next few years to include children under 16, with an acceptance rate of over 70%. The incidence of diabetes rose from 11.2 cases/100,000 in 1982-87 to 18.1/100,000 in 1989-91 (P=.0008). Classen attributes this highly significant increase to the late hepatitis B immunization.	Willis et al., 1997 (cited in PIDJ) question the published association between the hepatitis b vaccine and the development of IDDM in New Zealand. They analyzed the incidence of IDDM in children born before February 1988 to children born after this time. Their analysis was flawed for two reasons. First it assumed those born prior to 1988 did not receive hepatitis B vaccine. In fact there was a massive catch-up program in New Zealand with the hepatitis B vaccine originally given just to all preschool children (Gunn, 1989) but soon expanded so that all the children under the age of 16 received the hepatitis B vaccine, not just those born after 1988. The acceptance rates were estimated to be above 70% (Personal communications, Dr. Harry Nicholls, Senior Advisor for Communicable Diseases, Ministry of Health, Wellington, NZ). Thus children born in the 1970s and early 1980s received the hepatitis B vaccine. Second the incidence of IDDM differs depending on the age of the child in most countries including New Zealand, with fewer cases of IDDM occurring in ages 1-5 versus 10-14 (Scott et al., 1992) . Willis' analysis only proves that the incidence of IDDM is higher in older children (those born before 1988) than the

	very young children (those born after 1988). Petousis-Harris (ref. HE) admit that there was a rise of IDDM following hepatitis B vaccine in the North Island of New Zealand. They say an rise in IDDM was expected. There is a clear contradiction in the New Zealand Public Health Department's statements. First Poutasi (1996) denies there is a rise of IDDM in the North Island following the introduction of HepB. Once they had to admit a rise in IDDM occurred Clearly the rise was not expected or they would have stated that first.
7. Hib, Finland, 1983-87. Classen and Classen, "Clustering of Cases of Insulin Dependent Diabetes (IDDM) Occurring Three Years After Hemophilus Influenza B (Hib) Immunization Support Causal Relationship Between Immunization and IDDM", Autoimmunity, 35(4):247-53 (2002);[8] Classen and Classen, "Association between type 1 diabetes and Hib vaccine: causal relation is likely", BMJ 319:1133 (10/23/99) (Ex. 5C)	
This study had both prospective and retrospective aspects. All children born in Finland 10/1/85-8/31/87 (~116,000) were randomized to receive either (1) 4 doses of a Hib vaccine (at 3, 4, 6 and 18 mos.) or (2) one dose at 24 months. In addition, (3) the 128,500 children borne in children in the 24 prior months, who did not receive any Hib vaccine, were used as a historical control. Epidemiology: Comparing the treatment groups to the historical controls, it found that the cumulative incidence was significantly higher for the 4 dose	Karvonen et al., 1999 (ref. EV) concluded that the Hib vaccine was unlikely to cause IDDM. However their analysis was severely flawed They compared groups receiving 4 doses to 1 dose and groups receiving 1 dose to 0 doses. This analysis minimizes the difference and misleads the reader. Most objective researchers would compare the group receiving 4 doses to the group receiving 0 doses. Alternatively they would compare the combined vaccinated groups to the group receiving 0 doses. Both reach statistical significance.

[8] Previously made of record as unpublished manuscript.

group than for the control for the 0-7 (two-tailed), 2-7 (one-tailed), 5-7 (same), and (0-10)(same) age groups. The cumulative incidence of IDDM/100,000 in the 3 groups were 261, 237, 207 at 7 years and 398, 376, 340 at 10 years of age respectively. The relative risk at 7 years was 1.26. It was also significantly higher for the 1 dose group than the 0 dose group for the 5-7 (one-tailed) age group. See table 1. Prospective study: In addition, clustering of cases is seen when the cumulative incidence is plotted against the age at diagnosis. Such clustering is seen even when the two treatment groups are compared, see Fig. 1(a). The curves separate at about 39 months of age and then become parallel. Analysis of this cluster reveals that the curves separate by about 20 cases/100,000 during a span of about 6 months, with a relative risk of 2.25 (p=0.04), see P250, col. 1.	Note that both regimens are contrary to the teachings of the Classen application (first admin should be before 42 days after birth). The cumulative difference in cases IDDM/100,000 between those receiving 4 doses and those receiving 0 doses is 54 cases (P=0.013) at 7 years and 58 cases at 10 years (P=0.029) using a single tail Fisher test. The relative risk equals 1.26 at 7 years. The cumulative difference between those receiving 4 or 1 doses and those receiving 0 doses is 42 cases (P=0.016) at 7 years and 47 cases at 10 years (P=0.028). Karvonen et al. did not analyze the clustering of cases. Jefferson (ref. HP) questioned Classen's "unpublished reanalysis" of the Finnish data which Jefferson et al. presented at the NIH workshop. That data is now published in a peer-reviewed journal. Jefferson's own analysis is that published in Karvonen (ref. EV) and hence ref. HP adds nothing to ref. EV. The same is true for Bedford (ref. HD).

8. BCG, Southern India Sanjeevi, et al., Ann. N.Y. Acad. Sci. 958: 293-6 (2002)	
Sanjeevi examines the effect of BCG immunization on the incidence of diabetes in Southern India. Table 1 relates to the frequency of autoantibodies in BCG-vaccinated and nonvaccinated diabetic patients; of 137 diabetics (identified by GAD65 and IA-2 (CA512) autoantibodies), 86 were vaccinated with BCG **immediately after birth**, while the remaining 51 had not received BCG at all. Hence, based on Classen's work, it would be expected that BCG immunization would decrease the risk. This was indeed what Sanjeevi observed. The frequency of these autoantibodies was significantly (P <0.0005 for GAD65, <0.001 for ICA512) decreased in BCG-vaccinated diabetics (compated to those not vaccinated with BCG. (36% vs. 67% for GAD65, 19% vs. 43% for ICA512), see Table 1. Table 2 is limited to **type 1** diabetes patients. The frequency of the two antibodies was again significantly (P<.001) decreased decreased in the BCG vaccinated subjects (54% vz. 100% for GAD65; 23% vs. 62% for ICA512). Sanjeevi, who has no association with Classen, concludes that "BCG vaccination has an immunomodulatory role and is associated with decreased autoantibody positivity in south Indian diabetic patients, which is in conformity with the observations from animal models of autoimmune diabetes."	This a new reference. However, it should be noted that it is the first study specific to India.

<table>
<tr><td colspan="2">9. Anthrax, U.S. Armed Forces[9]
Institute of Medicine, The Anthrax Vaccine: Is It Safe? Does It Work? (March, 2002), available online from the National Academy Press,
http://www.nap.edu/books/0309083095/html/</td></tr>
<tr><td>Anthrax vaccine was given to 150,000 service members deployed for the Gulf War (1991). Later, DOD announced a plan for the mandatory vaccination of all U.S. service members. The program (AVA) began in March, 1998 with personnel sent to high-risk areas, such as South Korea and Southwest Asia. The vaccine is administered in a series of six subcutaneous injections. Obviously, the first administration was no earlier than the minimum age for military service.
The Classen claims are supported by the IOM data for vaccinated service members (Table G-1). Each member's pre-vaccination service time served as control for that member's post-vaccination service time. The data in Table G-1 was based, as Table 6-4 notes, on 738,382 person-years post-vaccination and 478,093 person-years pre-vaccination.
As shown in Table G-1, service members immunized with anthrax vaccine exhibited a significantly higher relative risk (3.46, 95% confidence limit of 1.51-7.90) of diabetes mellitus, post- vs. pre-vaccination. Table 6-4 says, "Of 843 diagnoses, adjusted RR significantly lowered for 12 diagnoses and</td><td>The report acknowledges that this study, with its "comparison of rates of hospitalization in the same individual before and after receipt of AVA removes many of the biases inherent in comparing groups vaccinated with AVA and groups not vaccinated with AVA." (P. 163).
However, it argues that for diabetes, "it is possible for the rate before vaccination with AVA to be artificially and differentially lower since those who had the disease and who had been hospitalized for it would be less likely to be deployed and therefore less likely to be vaccinated." (P. 163). It also asserts that since the ratio of the rate of hospitalization for diabetes before vaccination with AVA (in those ultimately vaccinated) to the rate of hospitalization for diabetes in those never vaccinated (0.12, CI 0.06-0.24)(P. 169) is much lower than the overall hospitalization rate ratio of 0.63(P. 166), that this"supports the conclusion that there is no increased risk attributable to AVA."(PP. 164, 168-9)</td></tr>
</table>

[9] This reference presents several studies, one of which reports that vaccination significantly increases the risk of diabetes, and others which do not. The favorable study is discussed here and the other studies in Table 2A.

significantly elevated for 15 (see Appendix G, Table G-1). Diagnoses with significantly elevated adjusted RR (95% CI) include ... Diabetes mellitus...." It was one of three diagnoses singled out by this table. The rise in diabetes rates post-immunization relative to pre-immunization was also found to be statistically significant in both men and women: "In examining the results stratified by sex, they are completely consistent. Yet there is only a 1 in 400 probability (0.05 x 0.05) that the results could be significant for both men and women independently by pure chance." P.168)	The pre/post immunization study was conducted **because** there was concern that it was inappropriate to compare vaccinated and unvaccinated personnel; the vaccine was given to personnel being deployed overseas, and these were considered to be generally healthier than the average military personnel. The finding that the pre-immunized group had a significantly lower hospitalization rate than the never-immunized group shows that this concern was **justified**. The latter were less healthy and therefore more likely to develop diabetes. Thus, an immunized vs. never-immunized study would tend to **underestimate** the risk of immunization. This effect would be especially prominent in the case of diabetes. diabetes is an age-dependent disease, with the number of cases increasing with age. Since immunization was essentially limited to troops deployed to high-risk areas overseas, the pre-immunization individuals would have been primarily personnel of combat age. In contrast, the never immunized group would have been primarily support personnel and, on average, substantially older. Thus, it is no surprise that the pre-immunized-to-never immunized hospitalization rate ratio is less for diabetes than for all hospitalization diagnoses collectively. One cannot fairly argue that the "never immunized" data is any indication that the

"pre-immunized" rate is artificially depressed.

Table G2 subdivided the post-immunization group into those hospitalized for diabetes within 45 days of immunization (RR 3.49, CI 1.39-8.79) and those so hospitalized more than 45 days after immunization (RR 3.44, CI 1.47-8.06). IOM argued that the similarity of these risk ratios suggested that there was no causal relationship (P.168). However, it is well accepted that type 1 diabetes arises from a chronically progressive autoimmune disease. The vaccine would be expected to accelerate the progression to a clinically recognized disease state. In those adults with extensive destruction of islet cells (from other causes) prior to immunization, diabetes could manifest itself within days or weeks, as in the 0-45 day group. In those adults with no prior damage to their islet cells, diabetes could develop as late as three or more years after immunization (see the "clustering" article, supra).

Indeed, the less than 45 day post-immunization data helps to refute IOM's never/pre argument. If the rise from pre to post had been the result of the artificial depression of the pre data, then why would there be such a rapid response to the immunization?

	IOM concedes that "finding an increased rate of occurrence of one or more adverse events must be considered a signal until proper review provides an alternative explanation." (P. 170)
10. military immunization, US vs. Europe Classen and Classen, "The safety of military immunization and the risk of insulin-dependent diabetes," Clin. Practice Alternative Med., 2:247-252 (2001)	
The US military vaccinates much more extensively than does the European military. Classen found that the risk of IDDM was significantly higher in the US military men than in conscripted European men age 20-35 (RR 1.6, CI 1.45-1.73). In countries where men, not women, are drafted, hence immunized by the military, the men have a significantly higher risk (RR 1.7, CI 1.53-1.84) of developing IDDM than do the women. In the US Navy, where both men and women receive vaccine, the incidence of IDDM is lower in men (RR 0.8, CI 0.64-0.97). The incidence of IDDM in the US Navy increased with age (and hence also with years of exposure to military immunization programs).	This is a newly reported finding.

III/3(3) Are there epidemiological studies by others which reach a different conclusion, and, if so, were they properly conducted?

Initially, the rejection relied solely on the PIDJ article. This article is a secondary source, that is, it does not report on the authors' own collection and analysis of epidemiological data, but rather reviews the analyses of others. Hence, the weight that it is given should be based on the merits (or lack thereof) of the studies upon which it relies. It bases its conclusions on several epidemiological studies:

BCG/Moulton (unpublished)
BCG/Parent (1997)/Canada
BCG/La Porta/China
various antigens/Blom Study/Sweden
various antigens/La Porta/global study
various antigens/Graves
HBV/Willis/New Zealand

The February 21, 2001 rejection, at pp. 7-8, also cited the following teachings as being allegedly supportive of PIDJ's conclusions:

DeStafano et al. (ref. DU)
EURODIAB substudy 2 study Group (ref. EB)
Graves, et al. (ref. EF)
Heijbel, et al. (ref. EI)
Hiltunen, et al. (ref. EL)
Jeffersen, et al. (ref. EQ)
Karvonen, et al. (ref. EV)
Bedford, H. (ref. HD)
Petouisis-Harris, et al. (ref. HE)
Dahlquist, et al. (ref. HK)
Jefferson T.O. (ref. HP)
Elliot et al. (ref. IJ)

Anonymous (ref. IN)

All of these studies and commentaries are analyzed in Table 2. Table 2A critiques epidemiological studies which reach conclusions unfavorable to applicant (Moulton, LaPorte, Parent, Blom, Graves, EURODIAB, DeStefano, Dahlquist, Heijbel and Elliot). The left column describes the study, and the right column critiques it. Table 2B discusses the remaining references, which either propose alternative interpretation of the data studied by Classen, or make conclusory statements regarding the studies of the others.

A general problem with these studies is that they were "underpowered" to detect effects of the magnitude reported by Classen. A study is designed to test a hypothesis, e.g., that immunization with a particular immunogen at a particular time affects the incidence of diabetes. In performing statistical significance tests, it is initially assumed that the study hypothesis is false, i.e., that there is no true difference between the larger populations represented by the study groups. This assumption is called the null hypothesis. Two types of error are recognized by statisticians.

A type I error arises when the study hypothesis is falsely accepted (and the null hypothesis falsely rejected). The significance, or P value, of a study is the probability of a type I error. It is conventional in the scientific community to consider P values of 0.05 or less to indicate the existence of a significant difference between the groups.

The converse error is a type II error; it arises when the study hypothesis is falsely rejected (and the null hypothesis falsely accepted). The smaller the number of individuals in the study, and the smaller the effect being looked for, the more difficult it is to produce data adequate to reject the null hypothesis.

The statistical power of a study is defined as the probability that a type II error will not occur. Most investigators would like the power to be at least 90%, that is, the probability of failing to demonstrate the statistical significance of a true difference to be 10% or less.

In a case-control study, the outcome is already known,

e.g., the cases are diabetics and the controls are normals. One retrospectively compares the relative exposure of the cases and controls to a hypothesized risk factor, such as Hib immunization. If there is a difference in exposure, implying a difference in relative risk, the statistical significance of this difference is tested.

The problem with the use of a case-control study to ascertain the riskiness of vaccination is that the percentage of both cases and controls who are vaccinated (the "uptake" or "utilization" of the vaccine) is high. If a case and a control received the same treatment, e.g., vaccination, they provide no information concerning differences between treatments. If most of the population has been immunized, most case-control pairs will be concordant, and the number of cases and controls necessary to give the study a given statistical power will be much higher.

Thus, for an unmatched case-control study, if we make the assumption that 90% of the controls were immunized, and that immunization produces a relative risk of 1.15, then, if the number of cases and controls is equal, the study would need 9,621 controls and 9,621 cases to reach a power of 80%.[10] If there were three times as many cases as controls (remember, diabetes is a relatively rare disorder), the same power (80%) would require 19,452 controls and 6,484 cases. To raise the power to 90% would necessitate 25,788 controls and 8,596 cases.[11] (See Exhibit to the most recent Classen Declaration.)

Most of the studies relied on by the Examiner are clearly underpowered. The few exceptions have other flaws, which are set forth in the table.

[10] Even if the relative risk were 1.30, to achieve 80% power would require 2,919 cases and 2,919 controls.

[11] These calculations use software implementing the formulae set forth in Fleiss, Statistical Methods for Rates and Proportions, pp. 38-45 (Wiley, 2d ed., 1981).

Table 2A: Critique of Epidemiological Studies Which Reached Conclusion Not Supportive of Applicant (Left col. description of study; right col. applicant's critique.)

1. Moulton (cited by PIDJ)	
PIDJ page 219 col. 2 quotes **unpublished** work by Dr. Larence Moulton to the effect that the rates of incidence of type I diabetes mellitus "in countries where BCG is routinely given at birth or at 1 to 3 months of age are generally lower than the rates where BCG is not given or given at>1 year of age." **This finding supports the present application.** However, PIDJ goes on to refer to "preliminary data from a multiple regression analysis" (presumably also by Moulton) which suggest that "these differences decrease after adjustment for distance from the equator, per capita gross national product, child mortality and per capita caloric intake". PIDJ also argues that "several other factors" could explain the observed differences in diabetes incidence, including "genetic differences in populations and increased exposure to immune modulating infections early in life in tropical climates". (page 219).	To say that the differences' "decrease" is not, of course, equivalent to saying that they vanish. Moreover, "preliminary data" is entitled to little weight, especially when it is unpublished and no detailed information (regression coefficients; R2) is given. A recent search on MEDLINE for "Moulton diabetes" found 11 articles meeting the search criterion, none of which appear to be the mysterious multiple regression analysis. Looking at Applicant's data (Appl., page 101), it is striking that Iceland (pertussis, no BCG) had a lower incidence (10.8) than the less northerly, equally developed, equally Caucasian study populations of England (16.4), Northern Ireland (16.6), Scotland (19.8), Denmark (21.5), Norway (20.8), and Finland (42.9) (late immunizations). Moreover, among Southern European states, Italy (6.8, 6.5, 30.2[12]); no pertussis or BCG, France (7.8; pertussis BCG <2 mo) and Portugal (7.5; same) scored lower than Spain (10.6, 10.9; pertussis, no BCG). It should be noted that the countries of Western Europe have relatively high and similar per capita GNP. **Conclusion: The PIDJ review of Moulton's analysis should not be given any weight.**

[12] The very high incidence of diabetes in Sardinian Italy can be explained on a genetic basis, see spec., page 92, lines 18-27.

2. all immunogens, Global LaPorte (cited by PIDJ)	
According to PIDJ, LaPorte presented data "demonstrating a global increase in the incidence of type 1 diabetes mellitus that cannot be explained by improved surveillance." However, PIDJ continues, "the incidence of type 1 diabetes has increased in countries with and without introductions of new vaccines into the immunization schedule." (219, col. 2,- 220, col. 1)	PIDJ does not cite any LaPorte publication as related to this passage. A MEDLINE search revealed Karvonen, et al., "Incidence of Childhood Type 1 Diabetes Worldwide," Diabetes Care, 23: 1516-26 (Oct. 2000). While this article acknowledges that "the incidence of type 1 diabetes appears to be increasing in almost all populations worldwide", it refused to rule out a surveillance effect: Whether this is a true increase resulting from changing lifestyle factors or is simply an improvement in case ascertainment is currently impossible to determine." (1524, col. 2). Also, LaPorte has not presented any of the particulars of this data, i.e., which vaccines were introduced in which countries at which times, what was the immunization protocol, and what was the rate of incidence of diabetes before and after these introductions or protocol changes. PIDJ does not consider whether there are differences in the rate of increase depending on whether a new vaccine had been introduced, or whether changes in old vaccines played any role. A new vaccine administered at birth could decrease incidence, shifting an old vaccine from birth to three months could increase it. Consequently, it is impossible to ascertain the merits of the conclusion stated by PIDJ. **This passage in PIDJ is not entitled to any weight.**
3. BCG-China LaPorte (cited by PIDJ)	
PIDJ declares that "Because BCG vaccine is given to almost all infants at birth in China, Dr.	PIDJ does not cite any LaPorte publication as related to this passage. A MEDLINE search revealed Yang et al.

4. BCG Canada Parent (1997) cited by PIDJ	
Parent (1997) studies the association between BCG immunization and the incidence of IDDM in Quebec. The Montreal paper contains two separate case control studies, series A and B. Series A pertains to children residing in a particular area of Montreal, born between 1970 and 1976, who were >6 years old at IDDM diagnosis. Controls were matched retrospectively. The authors report that 5 of 93 diabetics had received first BCG immunization when 1-12 years old, as compared to 124 of 2,903 controls (odds ratio of 1.26). Also,, 15 of the diabetics and 499 of the controls received first BCG immunization at "0 years old" (odds ratio 0.94) Series B contained 249 cases of IDDM (diagnosed from 1982-86, not more than 18 years old; residing in metropolitan Montreal) and 431 prospectively collected matched controls. The authors found 14 of 249 diabetics (31.8% of the vaccinated diabetics) had received first BCG immunization when 1-12 years old, versus 12 of 431 controls (Table 4)(18.4% of the vaccinated controls), yielding an **odds ratio of 2**. In contrast, 30 diabetics received first BCG immunization at "0 years old" (68.2% of the vaccinated diabetics), as compared to 53 of controls (81.5% of the vaccinated controls),	Use of the Series A data is problematic in that the controls were at least 10 years old (Parent, p.768, col. 3). Classen;s data indicated that the majority of the effect of BCG immunization of IDDM is within 4 years for a school age administration and within 7 years for a birth administration. Hence, the nature of the Series A data was such as would tend to underestimate the incidence of diabetes. Parent et al.'s original analysis is also of limited relevance here because it did not consider the effect of exact timing of the first dose of BCG vaccine on the development of IDDM. Sufficient data was not available to determine how many children immunized in the first year of life were actually immunized in the first month of life. However, re-analysis of cases and controls immunized starting after 1 year of life with the BCG vaccine indicates the vaccine, thus administered, is associated with an increased risk of IDDM. Classen has performed a stratified statistical re-analysis, comparing the incidence in children with BCG exposure starting after age 1 to the remaining children, in a dataset combining series A and B. The result was a relative risk of 2.3, with a P value (one tailed) of 0.019. **This is, of course, supportive of the present patent application**. See Classen et al., "Immunization with BCG vaccine starting after age 1 is associated with an increased risk of IDDM in Quebec" (unpublished, copy enclosed).

yielding an improved odds ratio (0.98:1). **This result, indicating that the timing of BCG immunization affects diabetes incidence, is consistent with applicant's epidemiological analysis.** Parent noted that "in series B, IDDM occurred at a more advanced age, on average, among vaccinated cases than among those who had not been vaccinated. Moreover, the proportion of cases who developed IDDM by age 5 years was much lower among cases who had been vaccinated at birth than among those who had not been vaccinated." Also, "control subjects were more likely (82% vs. 68%) to be vaccinated at birth than the cases". Parent saw no such difference in series A. Parent conceded that immunization with BCG at birth may have retarded the onset of diabetes. (770, col. 3)	
5. Various immunogens, Sweden Blom 1991	
This was a case control study of 339 recently onset diabetic and 528 referent children in Sweden, with the cases of diabetes being those in the 0-14 yr, age group reported 9/1/85-8/31/86. Table 3 shows the odds ratios and confidence intervals for vaccinated diabetic and referent children, for vaccination with tuberculosis, smallpox, tetanus, polio, measles, mumps, rubella, and a combined vaccine including diphtheria.	This analysis is likely to underestimate the effect of these commonly used vaccines on the incidence of IDDM because case control studies greatly underestimate the association when there is very high utilization of the vaccine.

6. HBV, Hib, polio, DPT; Colorado
Graves (ref. EE)

A small case-control study of the effect of immunization with HBV, Hib, polio and DPT; the cases were children enrolled in a prospective cohort study in Denver, Colorado. The study examined whether the cases and controls had received any HBV, Hib, Polio or DPT before 9 months, in particular, at birth, and the median age of the first such immunizsation.

The study group included 25 cases and 292 controls. No statistically significant differences in rates of incidence between cases and controls were found.

The study conceded that both the incidence of diabetes, and the number of different immunogens used in vaccination, have increased over the past 20 years. While the author concluded that changing the immunization schedule would not lower the risk of developing type 1 diabetes, the author hedged by saying that "further case-control studies would be valuable in addressing the lack of data on the effect of immunizations on the risk of developing type 1 diabetes."

This study had several limitations. First, it did not wait for diabetes to actually develop. Graves considered a positive reaction with "at least one autoantibody" to be indicative of diabetes, and it is well known that a single autoantibody has very low specificity for predicting the development of IDDM.

Second, Graves studied only 25 individuals with an autoantibody and 292 controls. Graves' study group has only found 5 antibody positive children who developed IDDM.

In summary, her study was too small, follow up too short, and markers too nonspecific to consistently make the findings seen by Classen for Finland

However, even with all these limitations, Graves found the Hib vaccine associated with an odds ratio of 1.64 which is even greater than the relative risk of 1.19 (166/140) found with the Hib vaccinated children by age 5 in Finland According to Graves, 72% of the 25 cases (receiving any Hib before 9 months; median age of first Hib was 2 months) and only 61% of the 292 controls developed autoantibodies. While this result, by itself, was not statistically significant ($p=0.275$), its results can be pooled with Applicant's studies which did reach statistical significance.

The HBV, polio and DTP studies were also underpowered (10-25 cases, 108-292 controls).

7. Nine immunogens, 7 European centers/countries EURODIAB (ref. TB)	
A seven center collaborative study looked for an association between vaccines and the development of IDDM (Paterson, 2000). The case-control study involved 900 diabetic children and 2,302 controls. The data was collected for children who registered for school in Austria (Vienna), Latvia, Lithuania, Luxembourg, Romania (Bucharest), United Kingdom (Leeds, Northern Ireland) in the period 1989-95 (varies from country to country). The authors calculated odds ratios for nine common vaccinations (tuberculosis, polio, tetanus, diphtheria, pertussis, rubella, measles, mumps, Hib) before and after adjustment for possible confounding variables (center, age group, breast feeding, birth weight, maternal age, jaundice at birth, asthma, and vitamin D supplementation). The unadjusted odds ratios ranged from 0.89 (pertussis) to 1.20 (tetanus), and the adjusted ratios from 0.75 (Hib) to 1.56 (tetanus). The best P value was 0.13, for the adjusted odds ratio (1.27) for rubella. The authors concede that "the hypothesis that early exposure to infections can reduce the risk of diabetes has advocates" (citing Rook et al. and Kolb et al.) and that "there is clear evidence to support it from animal models." They also concluded that early perinatal infections are risk factors for childhood onset of type 1 diabetes.	This study did not make any attempt to distinguish between early and late immunization. Since Applicant's thesis is that early immunization decreases the risk and late immunization increases it, this logically would be expected to blur the relationship between the timing of immunization and the risk of type 1 diabetes. The data shows the hemophilus vaccine was associated with a Relative Risk of 1.16 which is consistent with the statistically significant effect of the Hemophilus vaccine on the incidence of IDDM in a cohort study from Finland (Classen & Classen, 1999). Because of the high level of utilization of the Hemophilus vaccine, a large study group would be needed to detect an effect of this magnitude. The EURODIAB study was underpowered. The diphtheria, tetanus, measles, rubella and polio vaccines were also associated with an increased risk of IDDM though not statistically significant alone. Again, the high rate of uptake of the vaccine in both cases and controls made it unlikely that an effect would be seen with a study of this size. The combined effect of the vaccines was associated with a relative risk of 1.7.

However, they concluded that "vaccinations do not exert any major modifying effect on the risk".	
8. Hepatitis B, USA De Stefano (ref. DU)	
A US government funded study (DeStefano et al., 1997) analyzed data from three HMOs in the USA for about 160,000 children born 1991-95. It concluded that the hypothesis that HepB vaccination at "birth" (more accurately, 0-21 days after birth) decreases IDDM risk was not supported by the data. De Stefano was unwilling to rule out the possibility that hepB vaccination, particularly at older ages, may increase IDDM risk.	The reported relative risk (RR) was 1.3 for those first vaccinated at 0-21 days of age and 1.9 for those first vaccinated at eight weeks or later. Thus, later first immunization was associated with a higher RR, and any immunization increased risk. In a more recent paper (DeStefano, et al., Pediatrics, 108: __, Dec., 2001), the reported RR is 0.51 for those first vaccinated at 0-14d, 0.53 for 15-55d, and 0.86 for 56 or more days. Thus, while later first immunization was associated with a higher RR, any immunization decreased risk. In both studies, the calculated RR was not statistically significant. It is difficult to do case-control studies of vaccines in the US where there are so many vaccines given and there is variability in what is given when, leading to confounding effects. Children who received the hepatitis B vaccine at birth may have been more likely to receive other new vaccines like the Hib vaccine, the chickenpox vaccine, etc., which, depending on their timing, may have increased the risk of diabetes. (In contrast, in Europe, there is much more uniformity in immunization schedules for a given country at a given time.) DeStefano did not adjust for the possible confounding effects of other immunizations. While the RR observed in this study was not, by itself, statistically significant, De Stefano's data may be pooled with Applicant's New Zealand data, which showed a statistically significant increase in diabetes incidence

<table>
<tr><td></td><td>following HepB immunization.</td></tr>
<tr><td colspan="2">9. BCG, Sweden 1973-77, Dahlquist (ref. HK)</td></tr>
<tr><td>Dahlquist and Gothefors (1995) examined the effect of BCG vaccination in Sweden. Before 1975 all newborns were offered BCG vaccination in the first month of life. In view of the side effects of the vaccine, general BCG vaccination was halted on 1 April 1975. Since then, only high risk groups were given BCG. In 1976, only 0.6% were vaccinated, and in 1976-80, only 2%.

Dahlquist examined the cumulative incidence of childhood IDDM in Sweden in children 4-15 yrs old born in 1973-1977. There was no formal statistical analysis, but eyeballing the plot of cumulative incidence against age of diagnosis for the four cohorts, Dahlquist concluded that there was "clearly no significant difference".

Dahlquist's cohort data, taken or derived from the caption to his Fig. 1, was

1973 345 320.69 (107,582)
1974 329 302.75 (108,671)
1976 342 351.39 (927,327)
1977 320 336.49 (95,098)

(year, # cases, rate per 100,000, cohort size)</td><td>A reanalysis of the data (Classen and Classen, Diabetologia, 39:500-501 (1996)(ex. 5A) indicates that BCG immunization at birth was associated with a clinically significant reduction in IDDM.
Dahlquist et al. fail to consider the confounding effect of the discontinuation of the smallpox vaccine in 1976.The smallpox vaccine was administered in Sweden primarily at 2 months or 9 months of age as compared to the BCG vaccine which was administered at birth. Data from NOD mice and human ecological studies show that vaccines administered starting after 2 months of life increase the incidence of IDDM thus having the opposite effect of administering vaccines at birth (Classen & Classen, 1997). The Swedish data needs to be analyzed in a way to compensate for the confounding effect of the smallpox vaccine.
Swedish law until early 1976 required immunization with smallpox vaccine prior to the age of 5. Unfortunately good records on the acceptance rates in the birth cohorts are not available. Swedish public health officials have indicated that the smallpox vaccine was being increasingly withheld in anticipation of the discontinuation of the law, as it became apparent to physicians that the risk of children developing adverse responses from immunization exceeded the risk of being infected with smallpox. Data from the Netherlands showed this trend clearly. In the Netherlands the smallpox vaccine was given around 9 month of age and was mandatory by age 1 before the law was repealed on November 28, 1975. The</td></tr>
</table>

	acceptance rates by age 1 in the Dutch birth cohorts of 1970-1975 were 88%, 87%, 82%, 66%, 47%, and 9% respectively. **Table 1 of the reanalysis** examines the differences between the birth cohorts which received BCG, 1973- 1974, and those that didn't, 1976-1977. Dahlquist and Gothefors' analysis which ignores the effect of the smallpox vaccine is listed as assumption A. Three additional assumptions were considered. The most appropriate way to compensate for the confounding effect of the smallpox vaccine would be to compare the middle (1974 and 1976) cohorts (assumption C), If so, the difference in cumulative incidence between cohorts is then 48.64 cases/100,000, with a highly significant one tail P value of 0.0057. This is consistent with the effect of BCG at birth reported by Classen, 52.8 cases/100,000. Even if one compares 1973-74 with 1975-6 (assumption A), there is still a 32.22/100,000 difference, with 1 tailed P value of 0.0363.

10. DTP Sweden Heijbel (Ref. EI)	
The effect of the DTP vaccine on IDDM was studied in Sweden (Heijbel et al., 1997). The study involved comparing the cumulative incidence of IDDM in birth cohorts that received a DTP vaccine lacking an aluminum adjuvant (1977 and 1978 birth cohorts) to birth cohorts receiving a DT vaccine containing an aluminum adjuvant (birth cohorts 1980 and 1981). Both groups appeared to have a similar rate of IDDM.	The analysis was flawed because the MMR vaccine was started at about the same time that the pertussis vaccine was discontinued in Sweden. The 1977 and 1978 birth cohorts which received the pertussis vaccine did not receive the MMR vaccine at 18 months. The 1980 and 1981 birth cohorts which did not receive the pertussis vaccines but did receive the MMR vaccine. Thus the results indicate the pertussis vaccine had an effect similar to the addition of the MMR vaccine; the latter is consistently associated with a relative risk of approximately 1.2. Furthermore, based on the study it is not possible to distinguish the effect of the aluminum adjuvant from the pertussis vaccine. Therefore one can not make a conclusion on the effect of the pertussis vaccine on IDDM. It is likely that both the aluminum adjuvant and the pertussis vaccine increase the risk of diabetes because both are immune stimulants.
11. Various Immunogens, Auckland, New Zealand Elliott (Ref. IJ)	

This abstract reports informally on the incidence of type 1 diabetes in the Auckland area (North Island, New Zealand) over a 20 year period. The authors report "no change in vaccination program involving any one vaccine could be associated with a change in diabetes incidence although the total number of vaccines used could."	The problem with the North Island data is that the population is more transient, and the population has risen in the Auckland area, making the data less accurate than the South Island (personal communication R. Elliott). However, the trend is the same as with the South Island. Elliott's cohort data is set forth in Table 3B of Classen, "Scientific Evidence Proving Vaccines Cause Type I IDDM (June 2000)(of record). This notes that HepB immunization began in 1988, that the average incidence of diabetes in the 1977-87 cohorts was 9.8/100,000, and that the average incidence in the 1989-96 cohorts was 13.3, yielding a relative risk of 1.36. In view of the unreliability of Elliott's data, Applicant does not believe that it should be used to quantify the risk. Nonetheless, the increase carries the implication that HepB immunization, as practiced in Auckland, increases the risk of diabetes.

<table>
<tr><td colspan="2">12. Anthrax - U.S. Armed Forces
Institute of Medicine, *The Anthrax Vaccine: Is It Safe? Does It Work?* (March, 2002), available online from the National Academy Press, http://www.nap.edu/books/0309083095/html/</td></tr>
<tr><td>Anthrax vaccine was given to 150,000 service members deployed for the Gulf War (1991). Later, DOD announced a plan for the mandatory vaccination of all U.S. service members. The program (AVA) began in March, 1998 with personnel sent to high-risk areas, such as South Korea and Southwest Asia. The vaccine is administered in a series of six subcutaneous injections. Obviously, the first administration was no earlier than the minimum age for military service.
One of the IOM studies of this program, comparing rates of disorders post- and pre-immunization, has already been discussed.
I turn now to consideration of the other IOM studies.</td><td>A large study of hospitalized personnel (2,651 vaccinated; 151,609 unvaccinated) apparently did not find an increased RR for diabetes. However, as pointed out previously, the study was inherently flawed because it used unvaccinated personnel as controls. Since only individuals being deployed to high risk areas were vaccinated, and healthy individuals would be preferentially deployed, the unvaccinated personnel would tend to less healthy and more likely to develop diabetes, leading to underestimation of the risk of diabetes attributable to vaccination.
Two small studies both reported an increased RR for diabetes, albeit not statistically significant by themselves. The Air Combat Command Study (5,177persons) found a relative risk of 1.68 (0.20-13.9) for ambulatory care visit for diabetes (vaccinated vs. unvaccinated). The Army Aviation Epidemiology Study (3,356 matched pairs of vaccinated and unvaccinated air crew personnel) found a relative risk of 1.25 (0.34-4.66) of diabetes. While these studies were underpowered to detect diabetes risk of the magnitude expected as a result of Applicant's work, they were superior in design to the larger study because they used matched controls.</td></tr>
</table>

[13] This reference presents several studies, one of which reports that vaccination significantly increases the risk of diabetes, and others which do not. The favorable study is discussed here and the other studies in Table 2A.

Table 2B: Secondary Sources Cited by the Examiner

Secondary sources are those which do not present any new data or analysis of their own, but merely comment on the work of others.

PIDJ	see discussion of Moulton, LaPorte, Blom, Willis, Graves and Parent in Tables 1 and 2A
Hiltunen (ref. EL) Hiltunen et al. wrote a paper (Hiltunen et al., 1999) pertaining to vaccines and IDDM, claiming that there is no clear evidence that immunization is associated with insulin dependent diabetes (IDDM).	They simply failed to cite animal toxicity studies (Classen, 1996) and epidemiology studies (Classen & Classen, 1997) which show immunization starting after 2 months is associated with an increased risk of IDDM.
Karvonen, Cepaitis, Tuomilehto (ref. EV)	This is an alternative interpretation of the data listed previously as Hib/Finland/Classen and hence is discussed in Table 1.
Bedford (ref. HD) Bedford and Elliman (Bedford & Elliman, 1999) wrote "The workshop panel (May 1998, Johns Hopkins University) concluded that the analytical methods were incorrect. Furthermore, data were available from Professor Tuomilehto showing that follow up over 10 years showed no difference in the incidence of diabetes between children who had received one dose of vaccine and those who had received four doses. The workshop panel examined evidence from several sources and concluded that "there is no evidence that any vaccines have increased the risk of type 1 diabetes in animals or humans."	There was no consensus at the JHU meeting. Panel members at the meeting, were asked to sign a consensus statement refuting an link between vaccines and IDDM but they refused. With respect to the reference to Tuomilehto's data, this is the same Hib/Finland data presented in Karvonen (ref. EV), which is interpreted differently by Classen and by Karvonen. We have already explained why the comparison of the one dose and four dose regimens was inappropriate.

Jefferson,. (HP) In this 1999 letter to BMJ, Jefferson, Rabinovich, and Tuomilehto not only questioned Classen's analysis of the Finland data, see Karvonen (ref. EV), but also asserted that the conclusion of the NIH workshop, presented in June 1998, was that studies in humans do not indicate an increase in type 1 diabetes attributable to any vaccine or the timing of immunisation. "	There was no consensus at the NIH meeting; no vote was taken.
Jefferson (ref. EQ) This is a review paper (Jefferson & Demicheli, 1998) claiming that there is no evidence vaccines cause insulin dependent diabetes (IDDM).	This conclusion must be placed in context; Jefferson declared that "international analytical literature is insufficent and of limited coverage to shed light on the possible link between onset of IDDM and vaccination." So it is unclear why he thought he could state any conclusion. Jefferson's conclusion was seemingly based solely on epidemiological data, with no consideration given to animal studies. Having been published in 1998. it necessarily fails to consider the epidemiological data of Classen and Classen (1999), and later publications with similar findings. Jefferson does not provide any details of his analysis and hence it is unclear how Classen's earlier studies are weighted against that of Blom and Hejbel.
Willis PIDJ ref. 49	Not an independent study, but rather a critique of a Classen study. Hence, it is discussed above in that context.
Petousis-Harris (ref. HE)	not an independent study, but a critique of Classen's New Zealand study, see above.

CDC (ref. IN) This anonymous fact sheet is critical of Classen's animal and epidemiological evidence. With regard to the animal data, it argues that many of the animal experiments included anthrax, which is rarely used in infants and children, and more generally that there are uncertainties in extrapolating from animals to humans. It criticizes some of the epidemiological studies as related to vaccines not used, or only infrequently used, in the USA (smallpox, BCG). It also questions intercountry analysis as potentially affected by many factors. In response to Classen's HIB/Finland analysis, it argues that his results are "inconclusive because the exact number of children in each group is not known and the noted differences may not be statistically significant."	Classen's animal experiments were not limited to use of anthrax, and an immunogen can be given early just for its antidiabetic effect, not to control an infectious disease. The animal tests, moreover, are only part of the supporting evidence; they cannot be viewed in isolation from the human epidemiological data. Whether or not smallpox or BCG are used in the USA, it is relevant to the issue of utility whether the timing of administration of those immunogens has an effect on the incidence of juvenile diabetes. The CDC comments on Classen's Hib/Finland analysis are out-of-date; the Classen 2002 paper provides the number of children in each group, and shows the existence of a statistically significant effect.

In view of the deficiencies of these studies and commentaries, the Examiner has failed to establish either that the PTO should doubt the assertion that vaccines can protect against or increase the risk of diabetes at all, or the assertion that it is proper to extrapolate from efficacy against type I diabetes in mice to efficacy against type I diabetes in humans.

III/3(4) Are there epidemiological studies by others which support applicant and, if so, were they properly conducted?

We respectfully direct the Examiner's attention to Sanjeevi et al., which is one of the studies listed in Table 1. Dr. Classen did not participate in any way in this study, yet it confirmed his conclusions.

We also suggest that the Examiner consider our remarks on the IOM study in Table 1, which found a significantly increased risk in diabetes incidence in military personnel immunized against anthrax when post- and pre-immunization groups were compared. In that Table entry, we point out why IOM's attempt to explain away this finding (obviously, the government doesn't want to be accused of endangering the health of its military personnel) is unsatisfactory.

III/3(5) Can the studies, if contradictory, be harmonized?

The studies of Table 2A do not truly contradict those of Table 1. The studies of Table 1 show that early immunization decreases the risk of diabetes, and late immunization increases it. In either case, the effect is relatively modest (under 50%), and there is no doubt that other factors affect the risk of diabetes, too.

The studies of Table 2A were, in general, underpowered to detect, in a statistically significant way, effects of the magnitude inferrable from the studies of Table 1.

Nonetheless, if the effect is not real, it is surprising that the studies of Table 2A, almost without exception, report relative risks of greater than 1.0 for late immunization. (See the new Classen declaration.)

III/3(6) On balance, do the various epidemiological studies by applicant and others, duly weighted to account for the quality and relevance of the study, support the scientific plausibility of Applicant's claimed approach to reducing the risk of diabetes?

Several epidemiological studies have reported a statistically significant effect of immunization on diabetes. (see Table 1). Other studies (see Table 2A) have reported statistically insignificant effects which, however, are generally in the same direction (late immunization increases risk), and hence, if pooled, support the studies of Table 1. Hence, Applicant's claimed approach to reducing the risk of diabetes by first immunizing early rather than late is scientifically plausible.

The Food and Drug Administration, whose expertise in this area is considerably greater than that of the PTO, has promulgated 21 CFR 201.57(e), which provides that the labeling of a drug "shall be revised to include a warning as soon as there is reasonable evidence of an association of a serious hazard with a drug; a causal relationship need not have been proved."

It appears that the Examiner is insisting on **proof** of a **causal relationship**, and we think that improper under 35 USC §§ 101, 112 ¶1.

Issue III/4. What Weight should be given to Applicant's Animal Data as Evidence of Enablement in Humans?

PIDJ concedes at page 219, col. 1, full para. 2 that administration of BCG vaccine to infant BB rats protects against development of diabetes mellitus, citing Qin and

Singth, J. Autoimmun. 10:271-8 (1997).[14] PIDJ further concedes that FCA (which contains killed Mycobacterium butyricum) is protective, citing an apparently unpublished report from Dr. Noel Maclaren (a participant in the workshop). And PIDJ admits that the Classen articles (corresponding closely to the present specification) demonstrated that immunization of NOD mice with "anthrax and possibly other vaccines"[15] resulted in a reduced incidence of diabetes.

The PIDJ article cited (page 219, col. 2) applicant's experimental studies of DTP immunization in mice. It commented that the cumulative incidence in DTP animals was "about 75%, similar to control animals in most other studies" and that the incidence of diabetes in applicant's control animals was lower (about 25%) than expected.

One of Classen's control groups had a low incidence of diabetes because the animals were vaccinated at birth, just like the treated group. It has been confirmed by Noel McClaren (Ref. 5E) that immunization of NOD mice at birth can prevent the development of diabetes.

Other than this attack, PIDJ confines itself to the remark, "selective vaccines are protective against type diabetes in animals but the data in humans are inconclusive."

In view of the deficiencies of PIDJ, the Examiner has failed to establish either that the PTO should doubt the assertion that vaccines can protect against or increase the risk of diabetes at cell, or the assertion that it is proper to extrapolate from efficacy against type I diabetes in mice to efficacy against type I diabetes in humans.

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[14] The title of this article refers to NOD mice, not BB rats, according to citation 42 in PIDJ's references.

[15] Despite the "possibly", the evidence for the other vaccines (pertussis, diphtheria and tetanus) was actually stronger than for anthrax, but these other vaccines are standard childhood vaccines and hence it is politically sensitive for IVS to acknowledge a linkage between them and diabetes, even a favorable one.

MPEP §2107.02(c) specifically states that "data generated using in vitro assays, or from testing in an animal model or a combination thereof almost invariably will be sufficient to establish therapeutic or pharmacological utility". It is well settled that animal data (or even in vitro data) can establish the utility of a therapeutic method in humans if there is an accepted correlation between efficacy in the animal in question, and efficacy in humans. See In re Jolles, 206 USPQ 885 (CCPA 1980); Nelson v. Bowley, 206 USPQ 881 (CCPA 1980); Cross v. Iizuka, 224 USPQ 739 (Fed. Cir. 1985). The law does not requires that this correlation be perfect, merely that it give the researcher a reasonable expectation that a drug which does well in animal testing will be successful in humans.

The expectation exists here because:

(1) the specification establishes efficacy in NOD mice, and NOD mice are an accepted animal model of diabetes mellitus in humans;

(2) the method of the present invention was effective in a second species of animals, BB rats, which are likewise accepted as animal models of human diabetes mellitus; and

(3) the utility of the present invention in humans is made more believable by human epidemiological data.

It is now widely accepted by those skilled in the art that type I diabetes in humans responds similarly to immune intervention as does diabetes in NOD mice and BB rats. Diabetes in all three species is considered to be an autoimmune disease based on the presence of islet cell autoantibodies and strong genetic linkage between the development of diabetes and MHC genes (New England Journal of Medicine 314:1360-1368,1986; Diabetes Reviews 1:15-42,1993). Immunological events occurring in the first 2 months of life have been clearly shown to be responsible for the development of diabetes in NOD mice and BB rats. Similarly, recent human epidemiology data shows that immunological events occurring at birth have a profound effect on the development of human

diabetes. These events include maternal fetal blood group incompatibility as well as exposure to rubella virus and nitrates at birth (Diabetes Reviews 1:15-42,1993; Diabetologia 35:671-765,1992).

The concept of diabetes in humans responding similarly to diabetes in NOD mice is widely accepted. This has been justified by therapeutic experience. Clinical trials have shown that type I diabetes in humans can be prevented by immunosuppressants like cyclosporine when administered to prediabetics or newly diagnosed diabetics (Diabetes Reviews 1:15-42,1993). Immunosuppressants have a similar effect on NOD mice and BB rats (Clinical and Investigative Medicine 10:488-495,1987). The NIH recently embarked on a trial of screening up to 80,000 children to initiate a program of treating prediabetics with insulin immunotherapy, after a small phase I trial in humans supported results developed in NOD mice (Lancet 341:927-928,1993).

By reason of these findings, the art has often recognized the value of NOD mice and BB rats as models for diabetes in humans and has used these models to evaluate anti-diabetic therapies. The citations of Appendix 2 to the Amendment of March 25, 1999 hereto illustrate the degree of acceptance these models have earned.

As described in the Declaration attached to that Amendment, diabetes prone BB rats were immunized according to the method disclosed in the specification in order to show that the method of immunization could prevent diabetes in other species beside NOD mice.

BB rats spontaneously develop diabetes at an early age as is the case in NOD mice and humans. Many of the findings present in human type I diabetics and in NOD mice are found in BB rats leading experts to believe diabetes in BB rats is also a autoimmune disorder. Insulitis develops in the pancreas of BB rats before the onset of diabetes while antibodies develop to islet cells and possibly to insulin. Diabetes can be

prevented by neonatal thymectomy as well as administration during the prediabetic period of cyclosporine, anti-lymphocyte antibodies, or purified lymphokines like TNF. Genetic experiments show that diabetes is closely linked to the MHC class II genes in BB rat as it is in humans. Many older rats develop autoimmune thyroiditis that is casually related to the development of diabetes as occurs in humans.

BB rats have an immunologically distinct disease from the disease in NOD mice. Diabetes develops in approximately equal numbers of males and females in contrast to NOD mice where disease develops more commonly in females. The incidence of diabetes in BB rats is not affected by gonadectomy or the administration of androgens as occurs with NOD mice. In contrast to humans and NOD mice, BB/Wor rats, the most commonly used substrain of BB rats, are severely lymphopenic. They have a marked decreased number of mature T lymphocytes in peripheral blood, spleen and lymph nodes. The CD4+ subset is substantially reduced but the CD8+ subset is almost completely absent. Natural killer cells are relatively over expressed. Several review papers have been published on this model (Diabetes and Metabolism Reviews, 8: 9-37;1992).

BB rats were immunized with a combination of the anthrax and DTP vaccines (n=20) or nothing as a control (n=28). Groups contained approximately equal number of male and female rats. The vaccinated group was given the following dosing schedule: day 1 (.1ml,1:5); day 4 (.15ml, 1:5), day 11 (.15ml, 1:5), day 25 (.2ml, 1:5), day 39 (.2ml, 1:5), day 53 (.2ml, 1:5), day 61 (.2ml 1:2.5), and every 14 days for 3 more injections at approximately (.2ml, 1:2.5). Days of injection varied by one at times. The notation 1:5 means 1 part vaccine to 5 parts PBS. At 16 weeks of age 54% of the untreated rats had developed diabetes and or died compared to 20% in the vaccinated group. At 20 weeks of age 54% of the untreated rats had developed diabetes and or died compared to 25% in the vaccinated group. At 32 weeks the results were 54% versus 35%

respectively (graph I) which represents a 34% reduction in the incidence of diabetes. The difference between the two groups were statistically significant at 20 weeks (P=0.027). The findings that the method of immunization can prevent diabetes in both NOD mice and BB rats provides strong proof that methods of immunization presented in the specification have the ability to prevent chronic immune mediated diseases in mammals with very different genetic defects.

The Examiner has questioned the extrapolation from rodents to humans "because of the criticality of the age of administration of the immunogens and the difference in maturation rates between rodents and humans" (OA February 21, 2001, §6, para. bridging pp. 8-9).

The issue of maturation rates is discussed in the specification. It is not the overall maturation rate which is important, just the rate of maturation of the immune system.

The specification states at page 27, lines 15-23:

> The immune systems of mice and men mature at comparable rates, with both species capable of mounting immune responses to vaccine antigens by the time the recipients are several months old. A comparison of the experimental and epidemiological examples in this specification supports this conclusion. Subtle differences in the rates of development of the immune systems of mice and humans may be detected however using a broad range of assays including <u>in vivo</u> assays, <u>in vitro</u> assays, <u>in vitro</u> assays and phenotypic cell assays.

It then discusses the appropriate assays in detail, at page 27, line 24 to page 29, line 12, and concludes at page 29, lines 13-19:

> The present invention therefore can include administration of the immunogens to humans when said humans' immune systems are in a state of maturation and responsiveness comparable to that of mice or rats at the times indicated above, in

> such circum-stances as it would be less effective to administer those immunogens to humans at the same chronological ages as they were administered to mice or rats.

Mice develop <u>faster</u> than humans. If we give a dose of vaccine before 42 days of age in mice, and it reduces the incidence or severity of diabetes, then giving the same vaccine at the same time to humans should also be effective, because, at the same age, the human will be at an even <u>earlier</u> stage of maturation than the mouse. In our examples, the day of first administration was day 8 in Example 1, day 1 in Example 2, day 10 in Example 3, day 1 in Example 4 (rate), and day 1 in Example 5. Even day 8 in the mouse will certainly correspond to a very young human.

In view of the issue raised by the Examiner, Applicants added new claims (102, 103, 106, 148, 252-255) which refer to first administration to a human subject when the immune system of that subject is at a state of maturation comparable to that achieved prior to 42 days after birth in a mouse or rat. New claim 148 is a method claim based on claim 32 but reciting the maturation limitation of claim 102 in place of the simple 42 day limitation of claim 32.

Nonetheless, we have not made all claims directly or indirectly dependent on claims 102 and 148 because we do not regard the "maturation" limitation to be necessary. Our conclusions regarding the timing of the first administration are based, not just on data from animal models, but also on human epidemiology, e.g.:

<u>Favorable</u>	<u>Unfavorable</u>
BCG before 2 months	Pertussis, BCG at school age
smallpox at birth (P98, L18-22)	Pertussis, Hib, BCG at 3 months and at school age
	Hib or meningal polysaccharide at 3 months to 5 years (P93, L15-18)

See pp. 89-106 of the specification.

This data suggest that drawing the line at 42 days (~1.5 months) in human immunization is not unreasonable.

In response to Elliot's cautions concerning extrapolating from mice to humans (OA page 9, lines 1-7), on Aug. 17, 2001 we submitted a copy of Classen and Classen, "Clustering of cases of insulin-dependent diabetes (IDDM) occurring three year after Haemophilus influenza B (Hib) immunization support causal relationship between immunization and IDDM".[16] The mice (NOD) received hepatitis B vaccine (HepB) at days 3 and 28. The "vaccinated" group also received DTaP, Hib and inactivated polio vaccines at weeks 10, 16 and 22. The "control" mice received saline injections at weeks 10, 16 and 22 instead. The "vaccinated group" developed diabetes at a higher rate.

The NOD mice were compared with Finnish children receiving 0, 1 (~26 months) or 4 (3, 4, 6 and 18 months) of HBV, as well as, of course, the usual childhood vaccinations. The additional doses were associated with a higher incidence of diabetes.

Thus, vaccination of NOD mice at weeks 10, 16 and 22 (i.e, 2 to 5 months) has an effect similar to vaccination of humans at 3-18 months. Hence, the extrapolation of the age of administration seems supported by the data.

Moreover, Elliot's comments must be placed in context; NOD mice and BB rats are overwhelmingly popular as animal models of human diabetes. See pp. 39-40 of Appellant's first Brief; for more detail, see Appendix 2 to the Amendment of March 25, 1999. Similarly, MRL-lpr mice are accepted animal models of SLE, see Appendix 1 to the March 25, 1999 amendment.

Vertically transmitted viral infections appear to be associated with an increased risk of diabetes and other autoimmune disease. Mothers become infected with viruses such

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[16] At the time, this was an unpublished manuscript. It has since been published, Autoimmunity, 35(4):247-53 (2002), and a copy was filed on Oct. 18, 2002.

as the enterovirus and rubella virus when they are pregnant leading to an infection of the newborn and an increased risk of autoimmunity including IDDM later in life.

Vertically transmitted viruses have been shown to induce diabetes in both mice and humans. For mice, see Gaskins, et al., J. Clin. Invest., 90:2220-7 (1992) (retrovirus in NOD mice); Suenaga and Brown, Diabetes, 37:1722 (1988) (abstract); Serreze, et al. Diabetes, 37:351-8 (1988). In humans, see Dahlquist, et al., Diabetes Care, 22:364-65 (1999) ("enterovirus RNA has been detected early in pregnancy in mothers of children who later become diabetic in a higher frequency than that found in mothers of control subjects")[17]. If the immune systems of newborn mice were drastically different from the immune systems of newborn humans, this commonality would not have been observed.

It has been shown that immunization of newborns with vaccines after birth can protect against vertical transmission of viruses such as hepatitis B. Administration of antiviral drugs after birth can prevent vertical transmission of the HIV (AIDs) virus to newborns. It is also known that administration of interferons will stop the replication and spread of viruses. In view of applicant's disclosure and the knowledge of the art, it would be expected that early administration of an immune stimulant like a vaccine (which causes interferon release) would impede vertical transmission of viruses and thereby decrease the risk of IDDM attributable to such transmissions. This mechanism would be expected to function in humans as well as in mice or rats.

While there is certainly some controversy as to the precise role of immunogens in autoimmunity, the patent standard does not require that there be a consensus, merely

[17] The discussion of HIV and CMV in the paragraph bridging pp. 9-10 of the October 27, 1999 response was not directed to the maturation rate issue considered here, but rather to issue X below. Hence, the Examiner's comments on page 7 of the March 13, 2000 communication are not apropos.

that the disclosed use be <u>scientifically</u> <u>plausible</u>.

Issue III/5. Does Applicant Properly Rely on Evidence that Immunization can Increase the Incidence of a Chronic Immune-Mediated Disorder.

At page 9 of the February 21, 2001 response, the Examiner states:

> Regarding applicant's agreement that there are now a large number of reports indicating vaccines may cause chronic immune mediated disorders, exhibits 1E, 5G, 1A, 5H, 5E, and Classen references, applicant is reminded that the present methods are drawn to reducing the incidence or severity of chronic immune-mediated disorders, not the converse. Thus, the relevance of this line of argument is not apparent.

This is a "half-full" or "half-empty" argument. If conventional practice elevates the risk of CIMDs, changing that practice reduces it. One is the converse of the other. The specification contemplates: (1) altering the immunization schedule to reduce the risk of CIMD, <u>and</u> (2) giving warnings when the schedule cannot be changed (see page 7, lines 11-14 and page 59, lines 11-14). It also contemplates limiting conventional immunization to those at high risk for infection, see page 70, lines 12-16.

Our epidemiological data likewise covers both late immunization-induced increases in diabetes incidence, and early immunization-induced decreases in diabetes incidence.

Similar comments apply to the Classen and Classen declaration. In the November 5, 2001 office action with regard to the rejected claims, the Examiner only deigns to specifically address the Classen & Classen (C&C) citation. The examiner says that this reference teaches away from enablement of the present claims, "as it teaches a correlation between an increased risk of IDDM (a chronic immune-mediated

disorder) and immunization".

Plainly, the Examiner misunderstands the disclosure and claims. Applicant made two complementary teachings:

- early immunization decreases the risk of diabetes (see, e.g., P15, L2-7; P53, L18-23)
- late immunization increases the risk of diabetes (see, e.g., P20, L11-15; P57, L14-18).

The Classen & Classen citation is completely consistent with these teachings.

While it is true that the method claims are drawn to reducing, not causing, CIMDs, the cited references show that vaccine administration can affect the incidence or severity of CID. They then must be placed in the context of Applicant's experiments showing that timing determines whether the effect is beneficial or detrimental.

III/6. Is there a plausible mechanism of action as to how immunization can affect the risk of diabetes and, if so, what weight should be given to the existence of such a mechanism?

While disclosure of a mechanism of action is not required for enablement, the existence of a plausible mechanism of action makes a new therapeutic modality more believable.

At page 15, line 14 to page 16, line 8 of the specification, Dr. Classen declares

> Without intending to be bound by any theory, early administration of immunogens can cause the release of lymphokines that may accelerate the maturation of the immune system. The immunization may act in several ways including:
>
> A. Enhancing destruction of autoimmune prone cells in the thymus;
>
> B. Enhancing the flow of normal T-cells from the thymus;
>
> C. Causing peripheral elimination of autoreactive

T-cells that have escaped the thymus;

D. Causing the release of interferons which prevent infection with autoimmune causing viruses; and/or

E. Causing migration of macrophages into the area of administration as in an injection site and away from an vital organ like the islet cells of the pancreas. The invading macrophages have the ability to act as antigen presenting cells and induce an autoimmune response against the vital tissue.

In contrast, the late administration of an immunogen can cause the release of lymphokines which may act as growth factors enabling autoimmune inducing cells to grown.

Lymphokines (and other cytokines) are discussed in more detail at pages 37-39 of the specification. Interferon alpha is specifically mentioned at page 38, line 7. The mechanism by which immunization with a broad range of vaccines at birth prevents diabetes can be explained through the release of alpha interferon (or other lymphokines). Alpha interferon is an molecule made by macrophages when they are activated by an immunological challenge such as an infectious organism or vaccine. Alpha interferon is routinely used to treat patients with hepatitis and other viral infections because the molecule has strong and broad antiviral activity. Alpha interferon induced by immunization at birth can help prevent diabetes through the suppression of congenital or neonatal infections, also called vertical infections. Studies from Sweden and Finland have indicated that 27% or more cases of insulin dependent diabetes are linked to a vertical infection with Coxsackie B virus. See Dahlquist, et al., Diabetologia, 38:1371-3 (1995); Hyoty, et al., Diabetes, 44:652-7 (1995). This data is consistent with early reports linking the development of insulin dependent diabetes to congenital rubella infections. Ginspberg-Gellner, et al., Diabetologia, 27:87-9 (1984). Inhibition of these infections through nonspecific mechanisms, in particular release of alpha interferon following immunization at birth, explains why early

immunization is associated with a reduced risk for developing diabetes. This mechanism of action also explains why early immunization prevents diabetes in NOD mice since a congenital viral infection has been suggested as a cause of diabetes in the NOD mouse. Gaskins, et al., J. Clin. Invest., 90:2220-7 (1992); Suenaga, et al., Diabetes, 37:1722-6 (1988); Nakagawa, et al., Diabetologia, 35:614-18 (1992).

The late administration of alpha interferon to patients has been reported to cause insulin dependent diabetes. Alpha interferon and the alpha interferon inducer Poly I:C have been shown to induce diabetes in rodents as well, explaining why late immunization induces diabetes in rodents. The induction of diabetes by late immunization also can be explained through the release of alpha interferon. The mechanism by which alpha interferon can induce diabetes include damaging the islet cells and speeding up a smoldering subclinical autoimmune disease.

The ability of interferon to modulate diabetes by two pathways, prevention through inhibiting viral infections and induction through stimulating an autoimmune response, explains the importance of timing of first immunization.

It is well accepted that immune suppressants like corticosteriods can suppress most if not all autoimmune diseases. It is also accepted that immune stimulants like interferons can exacerbate almost all autoimmune diseases. The PDR gives specific contraindication not to give interferons to patients with autoimmune disease (PDR (1999) on Roferon). Vaccines induce interferons and would be expected to increase the risk of autoimmunity. Immune stimulation is a common pathway for exacerbating autoimmunity. A second common pathway for induction of autoimmune diseases is through vertical transmission of viruses. Interferon release following immune stimulation with vaccines would expect to prevent this (see below, and Classen, J.B., and Classen, D.C.: "Vertically transmitted enteroviruses and the benefits of

neonatal immunization" Diabetes Care 22(10):1760 (1999)).

It follows from the general effects of immune suppressants and immune stimulants on autoimmune diseases that there are common mechanisms at work.

The antidiabetic effect is not a specific immune response to a diabetes-associated autoantigens, because, e.g., diphtheria and tetanus are not known to cross-react with such an antigen. If the effect is not a specific immune response, it is reasonable to expect that it can be achieved with many different antigens.

Applicant's analysis is supported by Singh, et al., "Stimulation of the developing immune system can prevent autoimmunity", J. Autoimmunity, 14:15-22 (2000) (copy enclosed). According to Singh, "microbial infections have the capacity to regulate autoimmunity both positively and negatively". This is not surprising as infectious organisms present immunogens to the immune system. The autoimmune effect is then dependent on timing. Singh et al. urge, in the caption to Fig. 1, that "immunostimulation of the developing immune system in genetically susceptible individuals can prevent the development of autoimmunity by inducing regulatory T cells". Citing Gibbon et al. (1997), they declare that "infection in early life may be associated with a reduced risk of type I diabetes in humans".

They also acknowledge the implications of applicant's epidemiological data; i.e., that deliberate stimulation of the immune system by vaccination can decrease (early vaccination) or increase (late vaccination) the diabetes risk. Based on their own studies of BCG vaccination in cyclophosphamide treated NOD mice, they warn that "the window of opportunity for protection from type I diabetes following disease induction is relatively narrow". Hence, the timing of immunization is important; ideally, it should precede the exposure to an inducing agent.

It should be noted that the autoimmune effect of

vaccination is likely to be considerably greater than the autoimmune effect of infection. For example, vaccines often include potentiating agents (adjuvants), and exposure is typically to a large bolus of immunogen at one, rather than the more gradual exposure typical of a pathogen reproducing on the surface of a mucous membrane. The adrenal gland can increase production of corticosteroids, to suppress an autoimmune response, but this takes about three days, and hence is better suited to control of infection-induced autoimmunity than of immunization-induced autoimmunity. See Classen and Classem, "Vaccines and the risk of insulin-dependent diabetes (IDDm): potential mechanism of action, Medical Hypotheses, 57(5); 532-38 (2001).

Another recent paper which supports the inferred relationship between the timing of foreign antigen exposure and autoimmunity is Ownby, et al., "Exposure to Dogs and Cats in the First Year of Life and Risk of Allergic Sensitization at 6 or 7 years of age", 288:963-72 (2002) (copy enclosed). Asthma is a chronic immune-mediated disorder. The risk of developing asthma is related to allergic sensitization. Ownby, et al. found, in a prospective study, that exposure to 2 or more dogs or cats in the first year of life was associated with a significantly lower probability of subsequent allergic sensitization", even after accounting for possible confounding variables such as later exposure to dogs and cats.[18]

While we have not <u>established</u> a mode of action (OA sec. 3(e)), the existence and assertion of a <u>plausible</u> mode of action renders the asserted utility more believable, and hence is legally relevant.

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[18] It is possible that the effect is attributable to exposure to bacterial endotoxins associated with the pets, rather than to exposure to pet allergens, but this does not affect the relevancy of Ownby et al's work to applicant's claims, since it does not matter for our purposes whether the immunostimulant is a pet allergen or a bacterial endotoxin.

III/7. Has Applicant Overcome the Prima Facie case of enablement vis-a-vis diabetes in humans?

In re Marzocchi, 439 F.2d 220,223-4, 169 USPQ 367, 369-70 (CCPA 1971) states

> it is incumbent upon the Patent Office, whenever a rejection on this basis is made to explain why it doubts the truth or accuracy of any statement in a supporting disclosure and to back up assertions of its own with acceptable evidence or reasoning which is inconsistent with the contested statement. Otherwise there would be no need for applicant to go to the trouble and expense of supporting his presumptively accurate disclosure.

Assuming arguendo that the citation of PIDJ and other references, some of which are complied by PIDJ, establishes a prima facie case of nonenablement, the fact remains that applicant's analysis of PIDJ, applicant's animal data, applicant's epidemiological data, and the supportive data of others all supports the conclusion that the prima facie case has been rebutted and the rejection should be withdrawn.

Applicant has used five different, unrelated immunogens (anthrax, plague, diphtheria, pertussis and tetanus) in two different animal models (NOD mice, BB rats) to demonstrate that early immunization has a protective effect against diabetes (Exs. 1-4, see §7.3.1 of Brief).

MPEP §2107.02(d) states that "Office personnel should not impose on applicants the unnecessary burden of providing evidence from human clinical trials." Nonetheless, Applicant has also supplied human epidemiological data supporting his assertion of utility. This data revealed that standard childhood immunizations (i.e., later than when taught herein) against infectious disease increased the incidence of diabetes. It also indicated that early immunization with BCG and smallpox reduced the incidence of diabetes (although this effect was not recognized prior to the instant invention).

The epidemiology study described in example 101 of the

specification showed that the incidence of diabetes in western European countries was closely correlated with a country's vaccination schedule. Europe was chosen because in a relatively small geographic area there are many different countries that have different immunization schedules and the incidence of diabetes in the countries is known. The people in the western European countries have closely related racial backgrounds, diets, economic standards of living, and standards of health care. Eastern European countries of the former communist block were excluded because their standard of living and standard of medical care is not up to western levels.

The data correlating the incidence of diabetes to immunization schedule in western European countries is presented in Tables I-IV of this application.

The data in Tables I-IV, discussed in Example 101, substantiates the experimental animal findings. According to Table I, administration of vaccines after 2 months increases the incidence of diabetes while administration of vaccines at birth can prevent diabetes. The findings are highly statistically significant. Administration of the pertussis vaccine after 2 month of age explains the higher incidence of diabetes in group 3 compared to most regions in group 1. Administration of the BCG vaccine after 2 months of age explains the higher incidence of diabetes in Group 4 compared to group 3. Administration of the Hemophilus influenza vaccine after 2 months of age explains the higher incidence of diabetes in group 5 compared to 4. The ability of the BCG vaccine to protect against diabetes when administered at birth explains the lower incidence of diabetes in group 2 compared to most regions in group 3.

Temporal studies (Table II-IV) were done to show the incidence of diabetes changed in a rational way after the immunization schedule changed. Published reports, showing that diabetes in humans can be caused by transient immune

disturbances at birth, are also discussed in Example 101.

Additional epidemiological data supportive of Applicant's claims has been developed post-filing, and is summarized in Table 1 of this Brief.

All of the epidemiological data is evidence of efficacy in humans. In re Irons, 144 USPQ 351 (CCPA 1965) held that "historical" data could be used to establish utility.

We have already pointed out the deficiencies of the epidemiological analyses cited in PIDJ or other references (see Tables 2A and 2B of Brief); they stand rebutted.

Issue III/8. Is the specification enabling for protection against chronic immune disorders other than diabetes, and especially for protection against autoimmune diseases, in particular, SLE?

In paragraph 4 of the Classen Declaration filed March 25, 1999, data is presented which shows that the method of the present invention inhibits spontaneous autoimmunity in MRL/1pr mice. These mice, absent intervention, develop a disease which closely resembles the autoimmune disorder Systemic Lupus Erythematosus (SLE) in humans. Like SLE patients, the MRL/1Pr mice develop anti-DNA and anti-nuclear autoantibodies which can form immune complexes, which in turn can cause arthritis, dermatitis, and glomerulonephritis.

The MRL data is important not only because it is a good model for human SLE but because this autoimmune disease is both genetically, immunologically, and clinically very different from diabetes. Appendix 1 to the March 25, 1999 amendment summarizes a few references verifying both the similarity of the disease in MRL mice to SLE in humans and the clinical importance of the MRL model.

As described in the declaration, MRL/MpJ-lpr mice were injected either with a control (PBS) or with the anthrax/DTP combination, following an immunization schedule within the teachings of the present invention. At 15 weeks, 26.3%

control mice exhibited significant proteinuria (an accepted sign of glomerulonephritis), while only 7.7% of the vaccinated mice developed comparable levels of protein in their urine.

This data of course supports extending coverage from diabetes to SLE. However, because SLE and diabetes are so different, it also lends support to generic coverage of chronic immune-mediated disorders, or at least of autoimmune diseases.

Diabetes and SLE are quite different autoimmune diseases. Diabetes results from immune destruction of specific cells, the islet cells, which are present in the pancrease. The pathogenesis involves both antibodies and cytotoxic T cells. SLE does not involve the destruction of a specific cell type. Instead the autoimmune disease is against soluble antigens or antigens that are not cell specific such as DNA.
The Examiner relies on Boumpas as evidence that the underlying pathogenesis of SLE is not fully known. Even if this is accurate, that does not mean that SLE cannot be treated by immunological means. It is certainly known that SLE is an immunological disorder.

Applicants have shown that in a recognized animal model of SLE, early immunization reduces the incidence of SLE (Example 5). This was accomplished even though the underlying pathogenesis of SLE in the animal model is not fully known, either.

Empirical treatments are just as patentable as treatments which are the product of some elegant and complete theory of pathogenesis. Absent substantial reason to doubt the reliability of the MRL/MpJ-lpr mouse model --and Boumpas does not express such doubts-- the empirical success in mice renders the proposed human utility believable.

See more generally the discussion in sections 11 and 12 of Applicant's first Brief.

Since June 20, 2000, the Examiner has not specifically questioned enablement for CIMDs other than diabetes and SLE.

However, to the extent the Examiner accepts enablement for diabetes and SLE, but not for other CIMDs, we direct the Board's attention to the discussion on pp. 49-52 of the May 1, 2000 Brief.

It is well settled that the number of embodiments embraced by a claim is not the best measure of the difficulty of practicing it without undue experimentation. Disorders which are manifested through a common mechanism are likely to have a common cure or palliative. For example, a patient suffering from an allergic response may be given an antihistamine, regardless of the nature of the allergen. A particular immunosuppressant may be useful for treating a variety of autoimmune diseases.

Generally speaking, it is believed that people are genetically predisposed to develop autoimmune diseases later in life. While the diseases are polygenic, at least some of the predispositive genes must affect multiple diseases, as it is not unusual for several members of the same family to manifest different autoimmune diseases, see Amer. J. Human Genet., 38:170-87 (1986) (Multiple diagnosis in the same individual is also not uncommon, see above). These phenomenon are in part explained by the link between certain high risk genes and the development of autoimmunity, in fact a single gene may be associated with an increased risk of several different autoimmune diseases, see above.

It is also believed that the autoimmune response is environmentally triggered, as it does not occur in all predisposed individuals. Both pathogens and xenobiotic substances have been identified as potential triggers; in some cases, the same agent has been identified as a trigger for more than one autoimmune disease, see Lancet, July 17, 1982 at page 159.

The different "diseases" are characterized based on <u>which</u> tissues are adversely affected, and how. Because the <u>proximate cause</u> is always an immune response, it is probable

that one or more steps in the causal chain are common to all or most autoimmune diseases, implying that a common cure or palliative is feasible.

Therapeutic cytokines including interferons and interleukin 2 cause a significant number of recipients to develop autoimmune disease (see Physician Desk Reference). These cytokines are released naturally following the exposure to infectious agents and or vaccines.

Thus, a therapeutic intervention at the cytokine production or release stage could have a pervasive effect on autoimmune diseases.

Moreover, it is possible to use a "brute force" approach, such as administration of a nonspecific "anti-inflammatory" or "immunosuppressive agent." These are used in clinical treatment of autoimmune diseases today (see PDR). The use of these agents indicates that the diseases treated do have at least in part a common mechanism.

Applicant has postulated, in his specification, that interferons modulate diabetes, and that the administration of immunogens affect interferon release by a non-immunogen specific mechanism, as previously discussed in this Appeal Brief at pp. 31-33.

Many patents have been issued which claim treatment of a large class of diseases while only showing examples of treating a single disease. In the field of autoimmune diseases, the following patents come to mind:

i) U.S. patent 4,695,459 claim 3 (column 6 line 45) claims a method of treating multiple diseases in humans including multiple sclerosis, systemic lupus erythematosus, psoriasis, juvenile onset diabetes, Sjorgren's disease, thyroid disease, or myasthenia gravis. (These are chronic immune-mediated disorders). The specification only gave an example of treating EAE in mice.

ii) U.S. patent 4,710,380 claim 1 (column 5 line 47) claims a method of treating human or mammal subjects for

"disorders characterized by an hyperactive immune response". The term is similar to the term chronic immune mediated disorders used in our application because both encompasses rheumatoid arthritis, lupus, type I diabetes, and other autoimmune disorders (page 36 line 8). Patent 4, 710,380 contains only examples of rheumatoid arthritis patients being treated with its claimed method, however, its claim 1 encompasses all hyperactive immune responses.

Applicant has shown that early immunization reduces the risk of both diabetes and SLE --two unrelated immune disorders-- in suitable animal models.

Issue III/9. Is the Specification Enabling for use of Viral Immunogens to elicit protection against diabetes?

This question was last raised by the Examiner in the March 13, 2000 (pp. 6-7) and September 29, 1999 (pp. 8-9) advisory actions. We are not sure that this point is still maintained by the examiner; out of prudence, we will address it briefly.

Applicant showed experimentally that anthrax, plague diphtheria, tetanus and pertussis immunogens could be used to protect diabetes-prone mice from diabetes.

The immune system does not treat viral proteins any differently than it does bacterial proteins. That is why both viral and bacterial vaccines of at least partially proteinaceous character are known in the art.

It is not up to Applicant to elaborate upon the exact antigenic differences among plague, anthrax, diphtheria, pertussis and tetanus. It is up to the Examiner to prove, if he can, that these vaccines are so similar that Applicant's success with these vaccines is not properly extrapolated to vaccines based on other immunogens. Nonetheless, we wish to point out the separate classifications of the source organisms in the art:

anthrax (Bacillus anthracis)

plague (Yersina pestis)
diphtheria (Corynebacterium diphtheriae)
pertussis (Bordetella pertussis)
tetanus (Clostridium tetani)

Anthrax and tetanus are in the family Bacillaceae, but plague is in the family Enterobacteriaceae, while diphtheria is in the Actinomycetes. The affiliation of Bordetella is uncertain, but these bacteria are strictly aerobic coccobacilli.

It would therefore be reasonable to expect that the divergence in antigenic makeup among the exemplified immunogens is substantial, in which case generic coverage of immunogens --especially coupled with a functional limitation -- is justified.

The effectiveness of other immunogens is also suggested by Applicant's epidemiological data (Example 101 and Classen, et al., Infect. Dis. 6:449-54 (1997)), from which Applicant reasonably inferred that early immunization with BCG and smallpox vaccines reduces the incidence of diabetes, and that late immunization with BCG, Hemophilus influenza, hepatitis B, meningococci polysaccharide, measles, mumps and rubella immunogens can increase diabetes. (The latter also implies that early immunization with the same immunogens would decrease diabetes.)

Besides the variety of bacterial immunogens which have been shown to reduce the incidence of diabetes, several viral immunogens have also been shown to have this effect.

The efficacy of a smallpox vaccine, administered at birth, has been established by epidemiological evidence. See Spec., pages 97-99, and Classen, J.B., and Classen, D.C.: "Immunization in the first month of life may explain decline in incidence of IDDM in the Netherlands," Autoimmunity 31:43-45 (1999).

More recently, the applicant has shown that a hepatitis B virus vaccine, administered on days 3 and 28 from birth,

significantly protected NOD mice from the development of diabetes (see Declaration filed September 7, 1999).

With regard to the HBV data, the Examiner has argued that HBV is a vaccine of known efficacy. That misses the critical points.

First of all, it is clear that a viral immunogen can affect the incidence of diabetes.

Secondly, it is clear from the variety of immunogens that have this effect that the protective response must be non-specific, hence not limited to HBV.

The present invention is directed to methods of reducing the incidence of an autoimmune disease, by early and frequent administration of immunogens.

It can be seen from both the experimental studies and the epidemiological data that a variety of immunogens -- plague, anthrax, diphtheria, tetanus, pertussis, BCG, Hemophilus influenzae, hepatitis B, measles, mumps, rubella and smallpox -- can affect the development of diabetes, and that early administration of BCG, plague, anthrax, anthrax + DT, anthrax + DPT, hepatitis B and smallpox immunogens can reduce the incidence of diabetes.[19]

Table VI of the 1994 Classen Declaration (copy enclosed), filed in the parent case, compared the anthrax, plague, DT, pertussis, Hib, BCG, smallpox and MMR vaccines in terms of the nature of the vaccine. There are considerable differences. Only the pertussis and BCG vaccines have been shown to contain an immunogen that cross-reacts to an autoantigen associated with type I diabetes mellitus.

Under these circumstances, it is clear that the anti-diabetic response cannot be entirely immunogen-specific, as there is no common epitope in question which could be eliciting the response. A nonspecific immune response must play an important role.

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[19] The effect of early administration of the other immunogens noted is not yet known, but is readily determined.

In conclusion, extrapolation to other immunogens is proper for several reasons.

First of all, the agents applicant used or studied epidemiologically are very different, so, if they all have this effect on autoimmune disorders, other agents are likely to do so, too.

Secondly, Applicant presents a rational basis (lymphokine release) for expecting a general effect of this type (see pp. 15-16 of the specification and issue III/6 above).

Thirdly, the alleged unpredictability of the relationship between autoimmune disorders and microbial infections has nothing to do with the invention.[20] Applicant is not suppressing an autoimmune disorder by suppressing a specific causative infection. Applicant's Examples show an effect in infection-free mice and rats.

Potential immunogens which could elicit, if administered early in life, an anti-diabetic immune response are discussed in great detail at pages 33-36, 41-44, in the Examples, and original claims 3, 17 and 19.

Methods of screening immunogens for suitability are discussed at length at pages 53-75, and are further exemplified by Examples 1 to 4 of the specification.

The question of coverage of future vaccines (4(j)) is one considered and resolved during the prosecution of the parent application, Serial No. 08/104,529, now USP 5,728,385. The Examiner of the parent application said that Applicant could not specifically claim immunization against HIV, but could claim immunogens, implicitly including HIV, generically. That permitted a compromise, whereby Applicant received generic coverage of various immunogens, including HIV, but did not specifically claim HIV. The present Examiner would apparently limit Applicant only to those antigens already in use as

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[20] The effect of infection can be expected to be variable because the timing of the infection, and hence of the immune stimulus, is not controlled.

vaccines.

That position is entirely without justification when the claimed purpose of the immunogen is merely to protect against diabetes. There was no known relationship between diabetes and anthrax, plague, diphtheria, pertussis or tetanus. Hence, the antidiabetic effect plainly was not a specific response, and hence there is no reason to believe that an HIV immunogen would not work just as well.

We need to remind the Examiner that the specification is presumptively enabling (In re Marzocchi), and that evidence of enablement (animal data, or human epidemiological data) must be rebutted by more relevant evidence of non-enablement in order to overcome that presumption. See MPEP § 2164.04, 2154.05, 2164.07.

The claims are written so that inoperative embodiments are automatically excluded (note the "acting" limitation), and see MPEP § 2164.08 (b). Applicant's discovery is of the general advantage of early immunization. It is not Applicant's job to identify every possible vaccine in order to enjoy generic protection of his discovery. If another scientist later identifies a protective immunogen for HSV, HCV, HIV or CMV, and administers it before 42 days after birth to reduce the incidence or severity of diabetes, then that later scientist is profiting from Dr. Classen's discovery, and should pay tribute to it.

With regard to the effect of administering viral proteins, all viral proteins can elicit an immune response. Some elicit a protective response, others do not. Whether the response is protective or not depends inter alia, on (1) are there several strains of the virus and, if so, is the protein in question strain-specific, and (2) does the virus travel through the blood or by cell-to-cell direct contact.

With regard to HIV, CMV, and HSV in particular, immunogens are known for each. See Gringeri, et al., J. Hum. Virol. 1:293-8 (1998) (HIV-1 Tat protein); Lambert, et al., J.

Acquir. Imm. Defic. Syndr. Hum. Retroviral., 1a:451-61 (1998) (HIV gp120, gp160); Limsuwan, et al., Vaccine, 16:142-9 (1998) (gp120 depleted inactivated vines HZ321); Straus, et al., J. Infect. Dis., 176:1129-34 (1997) (HSV type 2 gpD and gbB); Adler, et al., Pediatr. Infect. Dis. J., 17:200-6 (1998) (live attenuated CMV Townestrain), copies of abstracts enclosed previously.

In view of the plethora of examples of potential immunogens, the diversity of the immunogens already known to affect diabetes, the plausibility of the proposed non-immunogen-specific mechanism (lymphokine release) by which the anti-diabetic effect is exerted, and the detailed presentation of the screening methodology, it is clear that one skilled in the art can, without undue experimentation, identify additional immunogens that can, by early administration, reduce the incidence of diabetes.

Therefore it does not appear that the disclosure is enabling only for the listed immunogens, as other immunogens would be expected to have an anti-autoimmune disease effect and to be identifiable without undue experimentation.

Issue III/10. Is the specification enabling for determining the effect of immunization schedules on the incidence or severity of immune disorders?

Finally, the Examiner refers to the alleged difficulties "calculating what dosage, method of administration, and frequency of administration" will "substantially induce an immune disorder" (see claim 2). It is, of course, as easy to determine whether an immunization schedule substantially induces a disorder as to determine whether it inhibits it. The test is the same; they are two sides of the same coin.

With regard to the issue of the determination of an effective immunization schedule, the PTO appears to have exaggerated the difficulty of this task. Applicants wish to call the Examiner's attention to the following considerations:

(a) it is routine in the art to conduct initial efficacy studies in mice and rats and to then scale-up to humans. This requires adjustment for differences in body weight, metabolism, development, etc. Such adjustments must now be deemed routine.

(b) immunization schedules are specifically suggested at pages 24-33 of the specification.

(c) dosages are discussed at pages 47-51 of the specification, and safe dosages are known for many of the contemplated immunogens. The epidemiology data indicates that the same doses given to prevent infections are also altering the risk of diabetes, therefor extensive testing to find the appropriate dose is not necessary.

(d) the human immunization schedules which resulted in favorable epidemiological effects on diabetes are known (see, e.g., table I, referring to vaccination with BCG at birth in 1988 in Ireland, France and Austria). Those dosages of other immunogens, such as pertussis, which, upon late administration, increased the incidence of diabetes are also known and presumably would still modulate diabetes (although more favorably) if given earlier.

The principal parameters of the immunization schedule are the timing of the first dose, the total number of doses, and the interval between doses.

The initial dosing date is addressed at page 25, lines 17-27; the total number of doses at page 25, line 28 to page 26, line 17, and the interval at page 26, line 18 to page 27, line 6. The interplay of these factors, as they affect the total number of doses within a given period, is discussed at page 27, lines 7-14.

Four specific immunization schedules are set forth in Table 5 and discussed at page 29, line 20 to page 30, line 30. These schedule can be characterized as follows:

Schedule	Dose, Initial	Doses, Total	Interval
1	w0	10	2w
2	w2	9	2w
3	w0	7	3w
4	w0	8	2wx3 3wx4

Note that schedule 4 is not entirely regular, but conforms to the practice discussed at page 26, lines 22-25.

The schedules in the Examples were

Schedule	Initial	Total	Interval
Ex. 1	8d	3	7dx1 14dx1
Ex. 2	1d	9	irregular (2d-2w)
Ex. 4	1d	10	irregular (3d-2w)

One skilled in the art would know the limitations of immunizing humans and would be able to design an vaccination schedule to perform the intended function. The frequency of immunization is limited by the frequency that individuals are willing to have a health official vaccinate their children. In Belgium in the 1960s, well baby care involved bringing the child to the doctor every 2 weeks until the child was 8 weeks old J. Royal College of General Practitioners 24:676-686, 1974).

It is respectfully urged that with the guidance of the recommendations and examples in the specification, a person of ordinary skill in the art can develop a safe and effective immunization schedule without undue experimentation. This conclusion is confirmed by paragraph 6 of the Classen Declaration.

In general, the response is expected to be increased by

administering the immunogen earlier, more often, at shorter intervals, and at higher doses. Therefore, if a preferred schedule is tried, and found less than optimal, one or more of the schedule parameters would be changed, i.e., starting earlier, giving more doses, reducing the dose interval, or increasing the size of each dose (or at least of the first dose). If the anti-diabetic response is satisfactory, but the anti-infectious disease effect (if sought), is unsatisfactory, the first dose may be given somewhat later. The practitioner may also wish to reduce the number of doses for economic reasons, or increase the time interval for the sake of patient convenience.

The systematic variation of a small number of quantifiable treatment parameters, so as to optimize the subject's response, is the very essence of routine practice.

With regard to the route of administration, several options are set forth on page 52. For each of the conventional pediatric immunogens, one or more accepted routes exist, and these would be used unless problems (not presently expected) are encountered. Most human vaccines are given intramuscularly.

8. <u>Definiteness Issues</u>

Claims 5, 6, 8-11, 16, 27-30, 34-47, 49-57, 77 and 86 are rejected for indefiniteness for "reasons of record", in OA §5. However, the only response made to Applicant's arguments was with respect to claims 6, 57 and 40, and in the case of 6 and 57, it merely reiterated the Examiner's conclusion. The rejection is therefore procedurally defective.

As a new rejection, claims 5, 30, 32, 67, 71, 73, 77-85, 144-152, 156 and 157 are rejected for indefiniteness in OA §6.

Reviewing OA §5, this appears to refer back to §4 of the February 21, 2001 rejection (rejecting claims 5, 6, 8-11, 16, 19, 27-30, <u>34-57</u>, 77 and 86), which in turn refers back to §5 of the June 20, 2000 rejection.

That office action raised the following issues:

(1) antecedent basis for "wherein for at least one such immunogen, the total dosage during the first 112 days after birth..." (claims 6 and 57);

(2) definiteness of total dosage "substantially greater" than that required for immunization against an infectious disease (claim 6);

(3) antecedent basis for instructions pertaining to the first 175 or 112 days from birth (claims 11 and 38);

(4) definiteness of recitations of immunogens by disease name (claim 19);

(5) definiteness of recitation of "herpes" without identifying a particular herpes virus (claim 19);

(6) definiteness of "cross-reacting molecule" (claim 19);

(7) definiteness of "substantially reduces the incidence" (claim 27);

(8) definiteness of range without explicit lower limit (claim 40);

(9) definiteness of "animal model" of diabetes or SLE (claim 48); and

(10) propriety of Markush format (claim 77)

The cancellation of claims 48 and 77 mooted (9) and (10). The November 5, 2001 office action withdrew the rejection of claim 19 for indefiniteness, so we assume that points (4)-(6) of the June 20, 2000 office action §5 are mooted. However, as to point (4), it appears that a similar rejection is being applied to certain other claims, see page 5, lines 6-10 and 15-19 of the November 5, 2001 office action.

Page 5, lines 3-5 of the November 5, 2001 action implies that claim 40 is rejected under 112/2 solely by reason of

"substantially" in base claim 27, i.e., point (8) is mooted.

Turning to the new indefiniteness rejection (OA §6) of the November 5, 2001 paper, this raises the following issues:

(a) definiteness of identification of immunogen as being of an organism which causes a particular disease, if the specification does not list the organisms which cause each disease; (claims 5, 30, 32, 67, 71, 73, 77-85, 144, 149, 150, 151, 152);
(b) definiteness of "substantially reduces the incidence" (claims 144, 145, 147);
(c) definiteness of total dosage during first 112 days after birth which is greater than that required for immunization against infectious disease (claim 146);
(d) definiteness of state of maturation" (claim 148);
(e) definiteness of range limitation lacking explicit lower limit (claim 148);
(f) definiteness of "flavivirus antigens" (claims 149-152);
(g) propriety of markush format (claims 150-152);
(h) dependency from cancelled claims (claims 156, 160); and
(i) improper dependency (claim 157).

The entry of substitute amendment "A" filed June 21, 2002 would moot (f), (g), and (i), and (h) at least as applied to claim 156.[21]

The entry of substitute amendment "C" addresses (a).

Entry of substitute amendment "B" would moot (e) (lower limit of one day) and also addresses (d).

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[21] Our intent was to cancel 153-258, see §2 of remarks, and the after-final rejection filed February 21, 2002. The supplemental amendment filed herewith cancels 159-258.

Thus, issues (3), (6), (8)-(10), and (f)-(i) are moot. This brief addresses the remaining issues, as follows:

IV/1	(1), (3)
IV/2	(c)
IV/3	-2
IV/4	(7), (b)
IV/5	(d), (4)
IV/6	(a)
IV/7	-5
IV/8	(e)

Issue IV/1. Is there antecedent basis for the limitations of claims 6, 11, 38 and 57?

The Examiner questions the antecedent basis for claims 6, 57, 11 and 38. In claims 6, 57 and 38, the questioned language is "during the first 112 days after birth". Claim 11 refers to "during the first 175 days from birth".

Base claim 32 recites administering one or more doses of each of one or more immunogens according to an immunization schedule. While the claim says that the first dose of the schedule must be administered when the mammal is less than 42 days old, measured from birth, it does not require that all immunizations be given before that time.

We realize that in dependent claims, one normally cannot recite "the X" unless "a/an X" has already been recited. However, to apply that rule to "during <u>the</u> first 112 days after birth" seems frivolous. Should we amend claim 32 to recite "during <u>a period which is from birth to a time</u> 112 days after birth"? Or, "<u>where the animal is alive 112 days after birth</u>, where during the first 112 days after birth"? Or is

the Examiner looking for something else? Similar comments apply to the other claims.

MPEP §2173.05(e) says that "the failure to provide an explicit antecedent basis for terms does not always render a claim indefinite. If the scope of a claim would be reasonably ascertainable by those skilled in the art, then the claim is not indefinite.... Inherent components of elements recited have antecedent basis in the recitation of the components themselves". The date of birth of a person is inherent to that person.

Reviewing the original rejection, we think that we may have misunderstood the Examiner's reasoning. We thought that the concern was with the word "the" before "first 112 days after birth". However, it may be that the Examiner simply thought that there was an inconsistency between reciting the "first 112 days after birth" in claim 6 and "prior to 42 days after birth" in base claim 32. If so, we can readily resolve the issue.

Claim 32 requires that for at least one immunogen, the <u>first dose</u> of the immunization schedule be administered when the mammal was "less than 42 days old, measured from both".

Claim 6 required that for at least one immunogen, the <u>total dose</u> administered under the schedule during the first 112 days after birth be substantially greater than that required for immunization against the corresponding infectious disease.

This language is readily harmonized. On page 32, Applicant presents several conventional immunization schedules, with DTP given at weeks 6, 10 and 14. Thus, there are 3 DTP doses in the first 112 days (16 weeks) after birth, and this is presumably all that is required for immunization against these diseases. On page 107, several preferred immunization schedules are given. In schedule 1, the first DTP dose is given in week 0, so the "first dose less than 42 days after birth" requirement of claim 32 is plainly

satisfied. DTP is given in weeks 0, 2, 4, 6, 8, 10, 12 and 14, a total of 8 doses in the first 112 days after birth. Even if we ignored the last dose, the total dose plainly exceeds that required for protection against diphtheria, tetanus and pertussis, see page 32, and hence satisfies claim 6.

Similar arguments may be made concerning claims 11, 38 and 57.

Issue IV/2. Is the language "total dosage greater than required for immunization against the infectious disease" (claim 146) definite?

Claim 146 requires a total dosage during the first 112 days after birth which is greater than that required for immunization against the infectious disease with which it is associated. The Examiner complains that the "specification fails to teach what diseases are associated with immunogens, fails to teach the metes and bounds of the dosage required for immunization against an associated disease, and fails to teach what is encompassed within a greater total dosage".

There are, of course, many disease for which associated immunogens are already known. For other diseases, the associated immunogens can be determined in the already conventional ways.

The present invention does not claim a method of determining the immunogens (or organisms) associated with a disease. Rather, once it is determined that an immunogen <u>is</u> so associated, and is protective against the infectious disease, it explains how to administer the immunogen while minimizing the risk of developing a chronic immune-mediated disorder.

Issue IV/3. If so, may applicant recite "substantially greater" (claim 6)?

With regard to "total dosage... substantially

greater than that required for immunization, "the Examiner asks "how much greater?" This is similar to the question asked by the Examiner in In re Mattison, 184 USPQ 485 (CCPA 1975) with respect to the meaning of "substantially increased efficiency". However, the CCPA did not consider "substantially" to be problematic:

> We are not persuaded by the board's reasoning that one skilled in the art would not be able to determine the scope of the claimed invention in terms of a specified percentage value. General guidelines are disclosed for a proper choice of the substituent Ep together with a representative number of examples.... Hypothesizing whether an increase in efficiency of 3%, 30%, or 300% is necessary for said increase to be classified as substantial is not determinative of the issue of whether the claims satisfy 35 U.S.C. 112, second paragraph.

The court reversed the rejection. Clearly, it is not necessary that all quantitative limitations of a claim be expressed as exact numbers.

Here, one may compare the total dosage under a schedule intended solely to immunize against a schedule intended solely to immunize against an infectious disease with the total dosage under a preferred schedule.

Three standard schedules, reflecting prior practice, are discussed on pages 31-32. Under these schedules. during the first 112 days (16 weeks) after birth, 3 administrations are given of each immunogen (D, T, P, polio, HepB, HiB). In the preferred schedules on pp. 107-108, schedule 3 called for five doses of HepB and six of DTP and Hib during the first 16 weeks. This clearly is considered a substantial increase in the total dosage. The preferred schedules, together with the stated purpose of the invention, provide a standard for judgment.

Issue IV/4. Is "substantially reduces the incidence" definite (claims 27, 144, 145, 147)?

The use of the relative term "substantially" has been repeatedly upheld when a suitable standard, such as a stated purpose, or representative examples, are disclosed. Andrew Corp. v. Gabriel Electronics, Inc., 6 USPQ2d 2010, 2012 (Fed, Cir. 1988); Seattle Box Co., Inc. v. Industrial Crafting & Packaging, Inc., 221 USPQ 568 (Fed. Cir. 1985); In re Mattison, 184 USPQ 485 (CCPA 1975).

It is clear that a rejection in incidence of 50%-75% would be considered substantial, see page 55, lines 17-20. However, this is not the minimum acceptable reduction.

In Ex. 1, the incidence of diabetes was reduced from 65% at 28 weeks in NOD mice, to 57.9% for the plague vaccine group and 42.1% for the anthrax vaccine group. See page 82, lines 21-27. That is a reduction of 7.1 percentage points out of 65, or 10.9%, for the plague vaccine, and 22.9 percentage points out of 65, or 35.2%, for the anthrax vaccine.

In Ex. 4, an experiment was conducted with another diabetes model, BB rats. The rats given anthrax + DTP had a diabetes incidence of 35% at 32 weeks, as compared to 54% for control rats. This was, as noted in the spec., a 34% reduction in incidence. See page 87, lines 15-22.

Other reductions in incidence may be extracted from the other examples, experimental and epidemiological, but the above seems sufficient to establish a standard.

Issue IV/5. Is the recitation of the "state of maturation" definite (claim 148)?

The examiner questions whether the state of maturation of the immune system (claim 148) is known in the art, or determinable without undue experimentation, for mammals other than mice or rats, or how it is to be correlated to that in a mouse or rat.

This claim is based on page 29, lines 13-19 of the

specification:

> The present invention therefore can include administration of the immunogens to humans when said humans immune systems are in a state of maturation and responsiveness comparable to that of mice or rats at the times indicated above, in such circumstances as it would be less effective to administer those immunogens to humans at the same chronological ages as they were administered to mice or rats.

The Examiner says that no markers of maturation are disclosed. This ignores the text at page 27, line 15 to page 29, line 12.

At page 27, lines 15-23, we begin:

> The immune systems of mice and men mature at comparable rates, with both species capable of mounting immune responses to vaccine antigens by the time the recipients are several months old. A comparison of the experimental and epidemiological examples in this specification supports this conclusion. Subtle differences in the rates of development of the immune systems of mice and humans may be detected however using a broad range of assays including *in vivo* assays, *in vitro* assays, *in vitro* assays and phenotypic cell assays.

The markers subsequently disclosed include:

(1) antibody titers in blood;

(2) DTH response;

(3) ability of T-cells to divide;

(4) ability of B-cells to divide;

(5) ability of immunocytes to secrete specific lymphokines;

(6) number of lymphocytes in the blood; and

(7) number of macrophages in the blood.

We do not understand why "correlation" should present any difficulties.

Issue IV/6. Is the identification of immunogens by the disease caused by the source organism proper? (claims 5, 30, 32, 67, 71, 73, 77-85, 144, 149, 150, 151, 152)

A person skilled in the art can readily determine whether an immunogen is associated with a particular disease just as such a person can determine whether a particular etiologic agent is causative of a particular disease. Hence while defining the immunogen by the associated disease is in some cases broader than defining it by the etiologic agent, it is not indefinite.

The Examiner asserts that the members of the recited Markus groups are not members of a recognized class because some members are "diseases" and others are "immunogens".

In fact, all of the members are "immunogens", whose source organisms were identified either directly or by reference to the diseases which they cause (and with which the immunogens in question are thereby associated). For some diseases, there is only one known pathogen, and for others, there are several. The common properties relating all of the immunogens are (1) they are immunogenic in mammals, and (2) they are mammalian pathogen-associated.

In answer to the Examiner's question, if several different organisms cause a recited disease D, then <u>any</u> immunogen associated with <u>any</u> of these organisms is contemplated as "D" immunogen.

Since herpes viruses are an art-recognized taxonomic group, and "herpes" is an art-recognized disease, it follows that reference to "immunogens of an organism causing herpes" is definite.

The Examiner has not formally responded to our explanation that all members of the questioned markush groups (presumably, those of claims 5, 30, 37, 56 and 77) are immunogens, but that some are identified by their source organism directly, and others by the diseases with which they are associated (and hence may embrace immunogens from more

than one organism). The wording of the objection suggests that this reasoning is accepted by the Examiner, so we do not understand why the rejection is maintained.

We are not aware of any authority which requires that an immunogen be identified by source organism rather than by disease.

Issue IV/7. Should the claims recite "herpes" or "herpes virus" (ditto)?

Claims 149 and 150 recite "herpes" in group (a), i.e., immunogens defined by the disease.

Applicant attempted to amend claims 149-150 to delete "herpes" from Markush group (a) and to insert "herpes virus" into Markus group (b). This was in response to the Examiner's comment that "the term 'herpes' is normally used to designate one of a specific group of viruses, rather than a disease per se". This February 21, 2002 amendment was refused entry, even though claims 151-152 already recited "herpes virus" in their Markush group (b).

In our opinion, "herpes" <u>is</u> a name of a disease, and "herpes immunogen" connotes an immunogen native to an organism which causes herpes. We have no doubt that the causative organisms <u>are</u> herpesviruses, but "herpes" is still the disease.

IV/8 Is claim 148 indefinite because it recites a range without an explicit lower limit?

The Examiner urges that the phrase, "the maximum interval between administrations is about two weeks or less" in claim 148 renders the claim indefinite, "as the lower limit of the claimed range cannot be ascertained.

The Examiner's attention is first respectfully directed to MPEP §2173.05(c)(II), page 2100-149, col. 2:

> In a claim directed to a chemical reaction process, a limitation required that the amount of one ingredient in the reaction

> mixture should "be maintained at less than 7 mole percent" based on the amount of another ingredient. The examiner argued that the claim was indefinite because the limitation sets only a maximum amount and is inclusive of substantially no ingredient resulting in termination of any reaction. The court did not agree because the claim was clearly directed to a reaction process which did not warrant distorting the overall meaning of the claim to preclude performing the claimed process. In re Kirsch, 498 F.2d 1389, 182 USPQ 286 (CCPA 1974).

The interval between administrations cannot be a negative number. Nor can it be zero, as then there is just one administration, not two. The situation is closely analogous to that of In re Kirsch.

Applicant has defined "dosing" in such a manner that the interval between dosings had to be at least 24 hours. At page 26, lines 8-11, the specification says:

> For the purpose of the appended claims, the administration of two different immunogens, or of two packets of the same immunogen, within a period of less than 24 hours, is considered a single dosing.

This implies that administrations 24 hours (1 day) apart are permissible and qualify as two separate dosings. Administrations less than 24 hours apart would be considered parts of a single dosing, and the "interval" limitation would not apply. Thus, by definition, the interval between dosings cannot be less than one day.

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Substitute Amendment "B", if entered, would have made explicit the implicit lower limit of one day (equivalent to 24 hours) and presumably would have mooted this rejection.

Respectfully submitted,

BROWDY AND NEIMARK, P.L.L.C.
Attorneys for Applicant

By:____________________
Iver P. Cooper
Reg. No. 28,005

<u>Enclosure</u>
-Singh, et al.
-Ownby, et al.
-Appendices 1 and 2

624 Ninth Street, N.W.
Washington, D.C. 20001
Telephone: (202) 628-5197
Facsimile: (202) 737-3528
IPC:lms
G:\BN\C\clas\classen1a\classen1a.pto 2ndappealbrief.wpd

APPENDIX 1

Summary of enablement rejections prior to the first Appellant's brief.

In the first office action, mailed October 2, 1998, the Examiner questioned whether the claims were enabled for subject matter other than just "immunizing against an infectious disease and against a chronic immune-mediated disorder in a mammal less than 96 months of age where the first dose begins within 42 days after birth wherein the immunogen is a combined anthrax vaccine and whole diphtheria, tetanus (i.e., DPT) composition"[22] (page 2, lines 14-18).

The wording of this passage implied that the rejection was not concerned with the choice of "mammal" (human v. mice v. rats) or the choice of "chronic immune-mediated disorder (diabetes v. other disorders). However, the body of the rejection questioned these choices, too.

In particular, the office action mailed October 2, 1998 argued that

(1) the art of preventing chronic immune-mediated disorders is a highly unpredictable [one, because]... the precise mechanisms by which the majority of autoimmune diseases remains unclear (page 2, lines 23-25)[23];

(2) the art considers the question of the earliest age at which to immunize [against an infectious disease] a difficult one to answer (OA page 3, lines 19-23)[24];

---

[22] "DPT" implies the presence of pertussis (P) immunogen, whereas "DT" is just diptheria and tetanus. The Examiner probably intended to recite "DT" since an anthrax + DT combination was administered in Exs. 2 and 3. However, those examples also looked at anthrax + DTP.

[23] Applicants do not purport to teach a method of "preventing" a chronic immune mediated disorder, only of <u>reducing</u> its incidence or severity.

[24] The question was whether to immunize later when the infants' immune system was more mature, but thereby running the risk that the infant would contract the disease in the interim.

(3) there are no vaccines for a number of the infectious viral conditions encompassed by the claims and hence no enabling disclosure of how to vaccinate against those conditions (page 6, lines 4-6)[25];

(4) the specification was limited to a teaching of the antidiabetic effect of a combined anthrax/DPT vaccine in NOD mice, MRL mice,[26] and BB rats (OA page 5, lines 13-15);

(5) there is no way to calculate what dosage, method of administration, and frequency of administration is required to prevent, for instance, HIV, HCV and HSV infection, (OA page 6, lines 4-6 and 11-13);

(6) there is no way to calculate what dosage, method of administration and frequency of administration is required to prevent "immunizing with an immunogen in such amounts and at such times as would substantially induce an immune-mediated disorder" (page 6, lines 13-17); and

(7) there is a dearth of guidance with respect to chronic immune-mediated disorders other than diabetes.

The final action mailed May 4, 1999 considered the issue of enablement in subsections 4a-4j. The arguments in these subsections may be briefly characterized as follows (where more than one issue is addressed in a single subsection, I list the issues in separate paragraphs, e.g., "a1" and "a2"):

a1) applicant does not elucidate the significant differences between anthrax and DPT;

a2) applicant has not demonstrated the same effect for "viral immunogens absent bacterial proteins and liposaccharides that has been demonstrated for the described bacterial antigens";

---

[25] The Examiner mentions hepatitis C virus (HCV), HIV, herpes viruses (esp. HSV-1 and HSV-2), adenoviruses, papoviruses, parvoviruses, and flaviviruses.

[26] As explained in the specification at page 81, lines 2-28, the MRL mice are a model for systemic lupus erythrematosis (SLE), an autoimmune disease which is "genetically, immunologically and clinically very different from diabetes".

b1) an alternative argument for the effectiveness of BCG is that it contains heat shock protein, a "known tolerogen";

b2) more generally, the epidemiological data presented is inconclusive;

c) because the mechanisms by which autoimmune disease arise is unknown, and their prevention is unpredictable, it is unclear whether the full breadth of the claimed invention would have a positive effect in treating autoimmune disease;

d) the relevance of the Classen declaration statement that only the pertussis and BCG vaccines have been shown to contain an immunogen that cross-reacts with an autoantigen associated with type I diabetes mellitus is not clear;

e) applicants' stated mechanism of action is only speculative;

f1) to the extent that applicants claim "a kit for use to protect a mammal against an infectious disease...", this suggests that the kit is used to elicit a [protective] immune response to the immunogen;

f2) it is unclear whether the mouse and rat data can be extrapolated to human infants because of the differences in growth and maturation rates;

g) autoimmune diseases are not known to share a common mechanism and hence would not have a common cure or palliative;

h) the two patents identified by applicants as pertinent to the number of examples required are legally irrelevant;

i) while the NOD mice and BB rats are accepted animal models for human diabetes, the extrapolation from rodents to humans is still in question because of the criticality of the age of administration of the

immunogen and the difference in maturation rates between rodents and humans; and

j) it would require undue experimentation to determine effective immunization schedules for a vaccine against an infectious disease (HIV, HCV, HSV) for which a protective immunogen is not yet known (or, to put it another way, for which the known antigens are not immunogenic).

In the Advisory Action mailed June 29, 1999, §10, the rejection of claims 5, 8, 10, 11, 30, 38, 49, 55, 60, 61-65, 72-100 for "recitation of protection" was "withdrawn". The Examiner reiterated point (3) above, concerning viral immunogens (at least as to HSV, HCV, HIV and (MV). She discounted the relevance of the Classen declaration regarding successful use of HBV, because HBV is "one of known effectiveness and immunogenicity". The Examiner also said that there was no "convincing evidence" that the antidiabetic effect was a nonspecific immune response, hence, one that could be achieved with many different antigens", but without any further discussion of the evidence presented.

The Examiner declared that the historical/epidemiological data was "inconclusive", again without explaining why. The Examiner also urged that "preventing autoimmune disease is higher unpredictable".

Finally, as to the extrapolation of data from rodents to humans, the Examiner argued that the time of onset of diabetes in rodents and humans is substantially different.

APPENDIX 2

5 (twice amended). The kit of claim 59 wherein one immunogen is provided which is not any of the following immunogens: a BCG, *Hemophilus influenzae*, *Streptococcus pneumoniae*, hepatitis A, hepatitis B, or *Neisseria* immunogen, or an immunogen of an organism which causes diphtheria, tetanus, pertussis, polio, measles, mumps, rubella, influenza, cholera, plague, varicella, rabies, typhoid or yellow fever.

6. The method of claim 32, wherein for at least one such immunogen, the total dosage during the first 112 days after birth is substantially greater than that required for immunization against the infectious disease with which it is associated.

8 (amended). The kit of claim 59 wherein, following such instructions, the first administration is when the mammal is less than 28 days old.

10 (amended). The kit of claim 59 wherein, following such instructions, the shortest interval between two successive dosings of at least one immunogen is les than 28 days.

11 (amended). The kit of claim 59 wherein, following such instructions, during the first 175 days from birth the longest interval between two successive dosings of at least one immunogen is less than 28 days.

16 (amended). The kit of claim 59 wherein, following such instructions, said mammal is a human.

27. A kit for use, prophylactically or therapeutically, to reduce the incidence or severity of a chronic immune mediated disorder, said kit comprising one or more containers, each container holding one or more pharmaceutically acceptable doses of one or more immunogens, said kit further comprising labeling indicating that the kit can be used to reduce the incidence or severity of a chronic immune-mediated disorder in a mammal, and instructions for the prophylactic or therapeutic use of said immunogens to reduce the incidence or severity of a chronic immune-mediated disorder in a mammal to which one or more doses of said immunogens are administered according to an immunization schedule set forth in said instructions, said immunogens, when so administered, acting to substantially reduce the incidence or severity of said chronic immune-mediated disorder, wherein said schedule, according to said instructions, calls for the first dose of an immunogen to be given before 42 days after birth.

28. The Kit of claim 27 where if the disorder is diabetes, the diabetes was not streptozotocin-induced.

29. The Kit of claim 43 wherein at least one immunogen other than a pertussis immunogen is administered.

30 (twice amended). The kit of claim 16 wherein said kit contains at least one immunogen selected from the group consisting of a *Hemophilus influenzae* immunogen, a BCG immunogen, a hepatitis B immunogen, and an immunogen of an organism which causes a disease selected from the group consisting of diphtheria, tetanus, polio, and pertussis.

32 (twice amended). A method of reducing the incidence or severity of a chronic immune-mediated disorder in a mammal which comprises administering to said mammal one or more immunogens, according to an immunization schedule by virtue of which the mammal receives, at, one or more pharmaceutically acceptable doses of said immunogens, said administrations resulting in an immune response in said mammal which substantially reduces the incidence or severity of at least one chronic immune-mediated disorder in the mammal,

the first dose of said immunization schedule being administered when the mammal is less than 42 days old, measured from birth,

where, if only one immunogen is administered according to said immunization schedule, that immunogen is one other than BCG, and, if said one immunogen is whole cell pertussis, the schedule is one other than a schedule of three doses at one week intervals, all given in the first month,

where, when all of the immunogens administered are selected from the group consisting of a BCG immunogen, *Hemophilus influenzae* immunogen and an immunogen of an organism which causes a disease selected from the group consisting of diphtheria, tetanus, whole cell pertussis, polio, hepatitis B, measles, mumps and rubella, at least one of the following conditions applies: (a) one or more immunogens are administered on at least three different dates prior to 42 days after birth, or (b) one or more immunogens are administered on at least three different dates, and the maximum interval between administrations is about two weeks, or less.

33. A method of reducing the incidence or severity of an immune disorder in a mammal which comprises administering to said mammal one or more immunogens, according to an immunization schedule by virtue of which the mammal receives, at specific times after birth, one or more pharmaceutically acceptable doses

of said immunogens, said administrations resulting in an immune response in said mammal which substantially reduces the incidence or severity of at least one chronic immune-mediated disorder in the mammal, the first dose of said immunization schedule being administered when the mammal is less than 42 days old, measured from birth,

where said immunogens are administered from a kit according to claim 27.

34. The kit of claim 27, where said kit is to be used to reduce the incidence or severity of an autoimmune disease, and said labeling so indicates and provides instruction for such use.

35. The kit of claim 27 wherein said labeling states that said kit is to be used to reduce the incidence or severity of systemic lupus erythematosus, and provides instruction for such use.

36. The kit of claim 43, at least one of said immunogens also acting to substantially reduce the incidence or severity of an infectious disease to which said mammal is susceptible, and said labeling so indicates, and provides instruction for such use.

37. The kit of claim 43, which includes at least one immunogen other than a BCG, diphtheria, tetanus, pertussis, polio, hepatitis A, hepatitis B, hemophilus influenza, measles, mumps and rubella, influenza, cholera, BCG, plague, pneumococcus, neisseria, varicella, rabies, typhoid or yellow fever immunogen.

38 (amended). The kit of claim 59, wherein, according to said instructions, for at least one such immunogen which elicits an immune response to one of said infectious diseases, the total dosage during the first 112 days after birth is greater than that required for immunization against the infectious disease with which it is associated.

39. The kit of claim 43, wherein, according to said instructions, the first administration when the mammal is less than 28 days old.

40 (amended). The kit of claim 27 wherein according to said instructions at least one immunogen is given in two or more dosings such that the shortest interval between two successive dosings thereof is at least one and less than 28 days.

41. The kit of claim 27, wherein according to said instructions at least one immunogen is given in two or more dosings such that the longest interval between two successive dosings thereof is less than 28 days.

43. The kit of claim 28 where the mammal is human.

44. The kit of claim 43 where said kit contains a killed vaccine.

46. The kit of claim 43 where said kit contains a live vaccine.

49. The kit of claim 16 where said labeling indicates that starting the first dose of immunization after 56 days after birth may not reduce said chronic immune mediated disorder or may increase the risk of said chronic immune mediated disorder.

50 (amended). The kit of claim 43 wherein, following such instructions, the first administration is when the mammal is less than 14 days old.

51 (amended). The kit of claim 43 wherein, following such instructions, the first administration is when the mammal is about 7 days old.

52 (amended). The kit of claim 27 wherein, following such instructions, the longest interval between two successive dosings is less than or about 14 days.

55. The kit of claim 16 in which at least one immunogen is a hemophilus influenza immunogen.

56 (twice amended). A method of reducing the incidence or severity of an immune disorder in a mammal which comprises administering to said mammal one or more immunogens, according to an immunization schedule by virtue of which the mammal receives, at, one or more pharmaceutically acceptable doses of said immunogens, said administrations resulting in an immune response in said mammal which substantially reduces the incidence or severity of at least one chronic immune-mediated disorder in the mammal,

the first dose of said immunization schedule being administered when the mammal is less than 42 days old, measured from birth,

where, if only one immunogen is administered according to said immunization schedule, that immunogen is one other than BCG, where, when all of the immunogens administered are selected from the group consisting of a BCG immunogen, a *Hemophilus influenzae* immunogen, a hepatitis B immunogen, and an immunogen of an organism which causes a disease selected from the group consisting of diphtheria, tetanus, pertussis, polio, measles, mumps and rubella, at least one of the following conditions applies: (a) one or more immunogens are administered on at least three different dates prior to 42 days after birth, or (b) one or more immunogens are administered on at least three different dates, and the maximum interval between administrations is about two weeks, or less, and

where one or more immunogens are administered on at least four different dates.

57. The method of claim 56 where one or more immunogens are administered on at least four different dates during the first 112 days after birth.

59 (amended). A kit for use to protect a mammal against an infectious disease to which a mammal is susceptible, said kit comprising one or more containers, each container holding one or more pharmaceutically acceptable doses of one or more immunogens, at least one of said immunogens acting to protect against said infectious disease when appropriately administered to said subject,

said kit comprising labeling containing information

(a) that the kit can be used to reduce the incidence or severity of a chronic immune-mediated disorder in a mammal, and providing instructions for the prophylactic or therapeutic use of said immunogens to reduce the incidence or severity of a chronic immune-mediated disorder in a mammal, said instructions stating that one or more doses should be administered according to an immunization schedule set forth in said instructions, said immunogens, when so administered, acting to substantially reduce the incidence or severity of said chronic immune-mediated disorder,

or

(b) that at least one immunogen of the kit, when administered according to one or more immunization schedules, may, can or does, or has been reported to, increase the incidence or accelerate the onset of a chronic immune-mediated disorder, or

(c) regarding any animal study or clinical study of the effect of any of said immunogens, or of any immunization schedule for any of said immunogens, on the incidence of a chronic immune-mediated disorder, or on the time of onset of said disorder.

60. The kit of claim 59 where (a) applies.

61. The kit of claim 59 where (b) applies.

62. The kit of claim 61, said labeling further comprising instructions for administering such immunogens so as to avoid such increase in the incidence or severity, or such acceleration in the onset, of said chronic immune-mediated disorder.

63. The kit of claim 59 wherein following such instructions the first administration is when the mammal is less than 14 days old.

64 (amended). The kit of claim 59 wherein following such instructions the first administration is at a time from birth to about 7 days after birth.

65. The kit of claim 59 wherein following such instructions the longest interval between two successive dosings is less than or about 14 days.

66. The kit of claim 43 where at least one of said immunogens is a pediatric immunogen.

67 (twice amended). The kit of claim 66 where said pediatric immunogen is selected from the group consisting of a BCG immunogen, a *Hemophilus influenzae* immunogen, a hepatitis B immunogen, and an immunogen which causes a disease selected from the group consisting of measles, mumps, rubella, diphtheria, pertussis, tetanus, and polio.

68. The kit of claim 43 where at least one of said immunogens is a nonpediatric immunogen.

71 (twice amended). The kit of claim 43 in which at least one immunogen is selected from the group consisting of a BCG immunogen, a *Hemophilus influenzae* immunogen, and an immunogen of an organism which causes a disease selected from the group consisting of anthrax, plague, tetanus, pertussis, diphtheria, hemophilus influenza and smallpox.

72. The kit of claim 16 where at least one of said immunogens is a pediatric immunogen.

73 (twice amended). The kit of claim 72 where said pediatric immunogen is selected from the group consisting of a BCG immunogen, a *Hemophilus influenzae* immunogen, a hepatitis B immunogen, and an immunogen which causes a disease selected from the group consisting of measles, mumps, rubella, diphtheria, pertussis, tetanus, and polio.

74. The kit of claim 16 where at least one of said immunogens is a nonpediatric immunogen.

77 (amended). The kit of claim 16 wherein at least one immunogen is selected from the group consisting of a BCG immunogen, a *Hemophilus influenzae* immunogen, and an immunogen of an organism which causes a disease selected from the group consisting of anthrax, plague, tetanus, pertussis, diphtheria, and smallpox.

78. The kit any of claims 59, 60, 61, 62, 96 97, 30, 49, 55, 74, 76, 77, 89-92, 98-100, 106, 114-17, 125 or 126 in which the disorder is an immune mediated cancer and where said mammal is human.

79. The kit any of claims 59, 60, 61, 62, 96 97, 30, 49, 55, 74, 76, 77, 89-92, 98-100, 106, 114-17, 125 or 126 in which the disorder is an autoimmune disease and where said mammal is human.

80. The kit of claim 79 in which the disease is a rheumatic disease or connective tissue disease and where said mammal is human.

81. The kit any of claims 59, 60, 61, 62, 96 97, 30, 49, 55, 74, 76, 77, 89-92, 98-100, 106, 114-17, 125 or 126 in which the disorder is a neurological disease and where said mammal is human.

82. The kit of claim 81 in which the disease is multiple sclerosis and where said mammal is human.

83. The kit any of claims 59, 60, 61, 62, 96 97, , 30, 49, 55, 74, 76, 77, 89-92, 98-100, 106, 114-17, 125 or 126 in which the disorder is asthma and where said mammal is human.

84. The kit any of claims 59, 60, 61, 62, 96 97, 30, 49, 55, 74, 76, 77, 89-92, 98-100, 106, 114-17, 125 or 126 in which the disorder is non-streptozotocin-induced diabetes and where said mammal is human.

85. The kit any of claims 59, 60, 61, 62, 96 97, 30, 49, 55, 74, 76, 77, 89-92, 98-100, 106, 114-17, 125 or 126 in which the disorder is systemic lupus erythematosus and where said mammal is human.

86. The kit of claim 59, said kit further comprising instructions for the use of an immunosuppressant to reduce the incidence or severity of chronic immune mediated disorder which might occur as a result of said administration of said immunogens in the absence of said immunosuppressant.

87. The kit of claim 59 which comprises said immunosuppressant.

88. The kit of claim 86 where said immunosuppressant is a glucocorticoid or a substance which induces the release of a glucocorticoid hormone.

90. The kit of claim 16 in which the disorder is one which develops at least one year after a vaccination.

91. The kit of claim 16 wherein at least one immunogen is a viral immunogen.

92. The kit of claim 16 wherein at least one immunogen is a bacterial immunogen.

93. The kit of claim 59 wherein at least one immunogen is a yeast, mold or plant immunogen.

94. The kit of claim 59 wherein at least one immunogen is an insect immunogen.

95. The kit of claim 59 wherein at least one immunogen is an immunogen of an allogeneic or xenogeneic animal.

96 (amended). The kit of claim 61 wherein the labeling indicates that the kit, depending on [when] <u>the immunization schedule according to which</u> one or more of said immunogens is administered, can or does increase the incidence or accelerate the onset of said disorder.

97 (amended). The kit of claim 61 wherein the labeling indicates that the kit, depending on [when] <u>the immunization schedule according to which</u> one or more of said immunogens is administered, may, can or does increase the incidence of said disorder.

98. The kit of claim 16 which includes at least one immunogen other than a pertussis immunogen.

99. The kit of claim 16 which includes at least one immunogen other than a BCG immunogen.

100. The kit of claim 16 where both (a) and (b) apply.

101. The method of claim 32 where at least one of said immunogens elicits an immune response in said mammal which recognizes an immunogen associated with an infectious disease to which said mammal is susceptible.--

--102. A kit for use, prophylactically or therapeutically, to reduce the incidence or severity of a chronic immune mediated disorder, said kit comprising one or more containers, each container holding one or more pharmaceutically acceptable doses of one or more immunogens, said kit further comprising a label for each container indicating the identity and amount of each immunogen in such container, and labeling indicating that the kit can be used to reduce the incidence or severity of a chronic immune-mediated disorder in a mammal, and instructions for the prophylactic or therapeutic use of said immunogens to reduce the incidence or severity of a chronic immune-mediated disorder in a mammal to which one or more doses of said immunogens are administered according to an immunization schedule set forth in said instructions, said immunogens, when so administered, acting to substantially reduce the incidence or severity of said chronic immune-mediated disorder, wherein said schedule, according to said instructions, calls for the first dose of an immunogen to be given before the subject's immune system arrives at a state of maturation comparable to that achieved at an age of 42 days after birth in a mouse or rat.

103. A method of reducing the incidence or severity of a chronic immune-mediated disorder in a mammal which comprises administering to said mammal one or more immunogens, according to an immunization schedule by virtue of which the mammal receives, at specific times after birth, one or more pharmaceutically acceptable doses of said immunogens, said administrations resulting in an immune response in said mammal which substantially reduces the incidence or severity of at least one chronic immune-mediated disorder in the mammal,

the first dose of said immunization schedule being administered when the mammal is less than 42 days old, measured from birth,

where said mammal is human, and at least one immunogen other than BCG or pertussis is administered before 42 days after birth.

104. The kit of claim 27 where the mammal is human and the disorder is an autoimmune disease.

105. The kit of claim 59 where the mammal is human and the disorder is an autoimmune disease.

106. The method of claim 103 in which at least one immunogen other than smallpox is administered before 42 days after birth.

107. The kit of claim 27, said kit further comprising a label for each container indicating the identity and amount of each immunogen in such container.

108. The kit of claim 59, said kit further comprising a label for each container indicating the identity and amount of each immunogen in such container.--

--109. The kit of claim 16 where said labeling indicates that humans with a family history of a chronic immune-mediated disorder may be at increased risk for developing that disorder after immunization.

110. The kit of claim 16 in which every immunogen is provided other than by a live vaccine.

111. The kit of claim 72 in which every immunogen is provided other than by a live vaccine.

112. The kit of claim 74 in which every immunogen is provided other than by a live vaccine.

113. The kit of claim 75 in which every immunogen is provided other than by a live vaccine.

114. The kit of claim 76 in which every immunogen is provided other than by a live vaccine.

115. The kit of claim 77 in which every immunogen is provided other than by a live vaccine.

116. The kit of claim 16 which is for use to protect

against at least two different infectious diseases, and provides at least one immunogen protecting against each of said diseases.

117. The kit of claim 16 which comprises both at least one pediatric immunogen and at least one non-pediatric immunogen.

118. The kit of claim 16 where said instructions provide for administering the first dose of at least one immunogen on or after 42 days after birth.

119. The kit of claim 16 wherein, according to said instructions, the first administration when the mammal is less than 28 days old.

120. The kit of claim 16 wherein, according to said instructions, the first administration when the mammal is less than 42 days old.

121. The kit of claim 30 where at least one immunogen is selected from the group consisting of a diphtheria, tetanus, polio, hepatitis B and hemophilus influenza B immunogens.

122. The kit of any of claims 66, 67, 68, 69, 70 or 71 in which every immunogen is provided other than by a live vaccine.

123. The kit of claim 43 wherein said kit contains at least one immunogen selected from the group consisting of a diphtheria, tetanus, polio, Hepatitis B, Hemophilus influenza b, pertussis, and BCG immunogen.

124. The kit of claim 43 wherein said kit contains at least one immunogen selected from the group consisting of diphtheria, tetanus, polio, Hepatitis B, and Hemophilus influenza b immunogens.

125. The kit of claim 43 which is for use to protect against at least two different infectious diseases, and providing at least one immunogen protecting against each of said diseases.

126. The kit of claim 43 which comprises both at least one pediatric immunogen and at least one non-pediatric immunogen.

127. The kit of any of claims 59, 60, 61, 62, 96, 97, 30, 49, 55, 74, 76, 77, 89, 91, 92, 98-100, or 106-117 in which the

disorder is one which develops at least one year after a vaccination.

128. A method of reducing the risk of a chronic immune-mediated disorder associated with immunization to protect against an infectious disease, comprising (1) determining the occurrence of at least one chronic immune mediated disorder in humans occurring during at least a one year time span after administering an immunogen according to one or more immunization schedules or determining the effect of timing of administering an immunogen on the development of a chronic immune mediated disorder, and (2) providing a kit according to claim 59 comprising at least one of said immunogens and labeling, said labeling indicating that one or more doses of said immunogen can be administered according to more than one immunization schedule or at more than one age set forth in said instructions, said immunogens, when so administered, acting to substantially protect against at least one infectious disease,

where administration according to different immunization schedules may have different effects on the incidence of said chronic immune mediated disorder;

and adhering to said warnings in said instructions may lead to a lower incidence of said chronic immune mediated disorder.

129 (amended). A method of protecting against an infectious disease which comprises providing a vaccine kit according to claim 59 comprising one or more immunogens protective against said disease, and instructions setting forth at least one immunization schedule for administering said immunogens, which, if followed, results in protection against such disease, said instructions stating that one or more immunogens can be administered according to more than one immunization schedule

and warning that administration according to different immunization schedules may have different effects on the incidence of a chronic immune mediated disorder;

so that adhering to said warnings in said instructions may lead to a lower incidence of said chronic immune mediated disorder.

130. In a method of vaccine packaging, wherein a vaccine is packaged with labeling providing directions for use, adequate warnings against unsafe dosage or methods or duration of administration, and information relating to side effects or to possible dangers to health when used as prescribed, recommended or suggested in the labeling, the improvement comprising: said labeling indicating that said vaccine may, can or has been reported to affect the incidence, in humans to whom it is administered, of a chronic immune mediated disorder.

131. The method of claim 127 where said labeling indicates that the timing of the first administration of said vaccine may, can or has been reported to affect said incidence.

132. The method of claim 128 where said labeling indicates that first administration of said vaccine before 42 days after birth may, can or has been reported to reduce the incidence.

133. The method of claim 127 where said labeling indicates that first administration of said vaccine on or after 42 days after birth may, can or has been reported to increase the incidence.

134. The method of claim 129 where said directions call for first administration before 42 days after birth.

135. The method of claim 130 where said package indicates individuals who have a mother, father or close relative with a chronic immune mediated disorder may be at risk for developing said chronic immune mediated disorder.

136. The method of claim 132 where said chronic immune mediated disorder is diabetes.

137. A method of human vaccine development and production which comprises

(a) screening a human vaccine for its effect, during

at least a one year time span after first administration, on the incidence of a chronic immune-mediated disorder when administered to humans in accordance with at least one immunization schedule, and

(b) labeling said human vaccine with labeling indicating that said human vaccine may affect the incidence of said chronic-immune-mediated disorder.

138. The method of claim 134 where said screening comprises comparing the incidence of the disorder in a treatment group of humans receiving said vaccine according to a first immunization schedule with the incidence of the disorder in a control group of humans.

139. The method of claim 135 in which the control group did not receive the vaccine.

140. The method of claim **135** in which the control group received the vaccine according to a second and different immunization schedule.

141. The method of claim 135 in which the difference in incidence between the treatment and control groups is calculated.

142. The method of claim 138 in which the statistical significance of said difference is calculated.

143. The method of claim 134 in which the vaccine is publicly distributed, accompanied by said labeling.--

144 (amended). A method of reducing the incidence or severity of a chronic immune-mediated disorder in a mammal which comprises administering to said mammal one or more immunogens, according to an immunization schedule by virtue of which the mammal receives, at, one or more pharmaceutically acceptable doses of said immunogens, said administrations resulting in an immune response in said mammal which substantially reduces the incidence or severity of at least one chronic immune-mediated disorder in the mammal,

the first dose of said immunization schedule being administered when the mammal is less than 42 days old, measured from birth,

wherein at least one immunogen is provided which is not any of the following immunogens: a BCG, a hepatitis A, a hepatitis B, a *Hemophilus influenzae, Streptococcus pneumoniae* or *Neisseria* immunogen, or an immunogen of an organism which causes diphtheria, tetanus, pertussis, polio, measles, mumps, rubella, influenza, cholera, plague, varicella, rabies, typhoid or yellow fever.

145 (new). A method of reducing the incidence or severity of a chronic immune-mediated disorder in a mammal which comprises administering to said mammal one or more immunogens, according to an immunization schedule by virtue of which the mammal receives, at, one or more pharmaceutically acceptable doses of

said immunogens, said administrations resulting in an immune response in said mammal which substantially reduces the incidence or severity of at least one chronic immune-mediated disorder in the mammal,

the first dose of said immunization schedule being administered when the mammal is less than 42 days old, measured from birth,

wherein at least one immunogen is administered on at least four different dates prior to 42 days after birth.

146 (new). A method of reducing the incidence or severity of a chronic immune-mediated disorder in a mammal which comprises administering to said mammal one or more immunogens, according to an immunization schedule by virtue of which the mammal receives, at, one or more pharmaceutically acceptable doses of said immunogens, said administrations resulting in an immune response in said mammal which substantially reduces the incidence or severity of at least one chronic immune-mediated disorder in the mammal,

the first dose of said immunization schedule being administered when the mammal is less than 42 days old, measured from birth,

wherein for at least one such immunogen, the total dosage during the first 112 days after birth is greater than that required for immunization against the infectious disease with which it is associated.

147 (new). A method of reducing the incidence or severity of a chronic immune-mediated disorder in a mammal which comprises administering to said mammal one or more immunogens, according to an immunization schedule by virtue of which the mammal receives, at, one or more pharmaceutically acceptable doses of said immunogens, said administrations resulting in an immune response in said mammal which substantially reduces the incidence or severity of at least one chronic immune-mediated disorder in

the mammal,

the first dose of said immunization schedule being administered when the mammal is less than 42 days old, measured from birth,

wherein at least one immunogen so administered is one other than pertussis, and a plurality of doses of that immunogen are administered.

148 (new). A method of reducing the incidence or severity of a chronic immune-mediated disorder in a mammal which comprises administering to said mammal one or more immunogens, according to an immunization schedule by virtue of which the mammal receives, at one or more pharmaceutically acceptable doses of said immunogens, said administrations resulting in an immune response in said mammal which substantially reduces the incidence or severity of at least one chronic immune-mediated disorder in the mammal,

the first dose of said immunization schedule being administered before the mammal's immune system arrives at a state of maturation comparable to that achieved at an age of 42 days after birth in a mouse or rat,

where, if only one immunogen is administered according to said immunization schedule, that immunogen is one other than BCG, and, if said one immunogen is whole cell pertussis, the schedule is one other than a schedule of three doses at one week intervals, all given in the first month,

where, when all of the immunogens administered are selected from the group consisting of BCG, diphtheria, tetanus, whole cell pertussis, polio, hepatitis B, hemophilus influenza, measles, mumps and rubella immunogens, at least one of the following conditions applies: (a) one or more immunogens are administered on at least three different dates prior to 42 days after birth, or (b) one or more immunogens are administered on at least three different dates, and the maximum interval between administrations

is about two weeks, or less.

149 (amended). The kit of claim 68 in which said nonpediatric immunogen is

(a) an immunogen of an organism which causes a disease selected from the group consisting of anthrax, plague, encephalitis, meningitis, typhus, typhoid fever, Lyme disease, cholera, leprosy, varicella, dengue, influenza, herpes, rabies, toxoplasmosis, coccidiomycosis, schistosomiasis and malaria, or

(b) an immunogen selected from the group consisting of *Streptococcus, Staphylococcus, Neisseria, Escherichia coli, Shigella, Leishmania,* cytomegalovirus (CMV), respiratory syncytial virus, Epstein-Barr virus, herpes virus, parainfluenza virus, rotavirus, adenovirus, human immunodeficiency virus (HIV), hepatitis A virus, and NonA NonB hepatitis virus immunogens.

150 (amended). The kit of claim 74 in which said nonpediatric immunogen is

(a) an immunogen of an organism which causes a disease selected from the group consisting of anthrax, plague, encephalitis, meningitis, typhus, typhoid fever, Lyme disease, cholera, leprosy, varicella, dengue, influenza, herpes, rabies, toxoplasmosis, coccidiomycosis, schistosomiasis and malaria, or

(b) an immunogen selected from the group consisting of *Streptococcus, Staphylococcus, Neisseria, Escherichia coli, Shigella, Leishmania,* cytomegalovirus (CMV), respiratory syncytial virus, Epstein-Barr virus, herpes virus, parainfluenza virus, rotavirus, adenovirus, human immunodeficiency virus (HIV), hepatitis A virus, and NonA NonB hepatitis virus immunogens.

151 (amended). The kit of claim 43 in which at least one immunogen is

(a) an immunogen of an organism which causes a disease selected from the group consisting of measles, mumps, rubella,

diphtheria, pertussis, tetanus, anthrax, plague, encephalitis, meningitis, pneumonia, typhus, typhoid fever, Lyme disease, cholera, leprosy, influenza, varicella, rabies, dengue, toxoplasmosis, coccidiomycosis, schistosomiasis, and malaria, or

(b) an immunogen selected from the group consisting of BCG, *Hemophilus influenza*, hepatitis B virus, polio virus, *Streptococcus, Staphylococcus, Neisseria, Escherichia coli, Shigella, Leishmania*, cytomegalovirus (CMV), respiratory syncytial virus, Epstein-Barr virus, herpes virus, parainfluenza virus, rotavirus, adenovirus, human immunodeficiency virus (HIV), hepatitis A virus, and NonA/NonB hepatitis virus immunogens.

152 (amended). The kit of claim 16 in which at least one immunogen is

(a) an immunogen of an organism which causes a disease selected from the group consisting of measles, mumps, rubella, diphtheria, pertussis, tetanus, anthrax, plague, encephalitis, meningitis, pneumonia, typhus, typhoid fever, Lyme disease, cholera, leprosy, influenza, varicella, rabies, dengue, toxoplasmosis, coccidiomycosis, schistosomiasis, and malaria, or

(b) an immunogen selected from the group consisting of BCG, *Hemophilus influenza*, hepatitis B virus, polio virus, *Streptococcus, Staphylococcus, Neisseria, Escherichia coli, Shigella, Leishmania*, cytomegalovirus (CMV), respiratory syncytial virus, Epstein-Barr virus, herpes virus, parainfluenza virus, rotavirus, adenovirus, human immunodeficiency virus (HIV), hepatitis A virus, and NonA/NonB hepatitis virus immunogens.

159 (new). The method of claim 153, wherein for at least one immunogen in at least one schedule, the total dosage during the first 112 days after birth is substantially greater than that required for protection against the infectious disease with which it is associated.

160 (new). The method of claim 1 wherein in at least one schedule at least one immunogen is administered by a route other than intravenously.

161 (new). The method of claim 153 wherein in at least one schedule at least one immunogen is administered subcutaneously, intradermally, or intramuscularly.

162 (new). The method of claim 153 wherein in at least one schedule at least one immunogen is administered other than as an immunogen of a live vaccine.

163 (new). The method of claim 153 wherein in at least one schedule at least one immunogen is not a BCG immunogen.

164 (new). The method of claim 153 wherein at least one immunogen of said subject immunization schedule which was also included in said lower risk schedule is one other than a pertussis immunogen.

165 (new). The method of claim 153 wherein the incidence of the disorder in said groups is compared.

166 (new). The method of claim 153 wherein the method is part of a production process to test vaccine lots for efficacy or safety.

167 (new). The method of claim 153 wherein the method is part of a development process or clinical trial of a vaccine to test a vaccine for safety or efficacy.

168 (new). The method of claim 153 wherein said mammals are human.

169 (new). The method of claim 153 wherein said mammals are rodents and diabetes has not been chemically induced by

streptozotocin.

170 (new). The method of claim 153 wherein said mammals are NOD mice or BB rats.

171 (new). The method of claim 153 wherein the comparison of (I) is prospective.

172 (new). The method of claim 153 wherein said mammals are randomized in said groups.

173 (new). The method of claim 153 wherein at least one said groups receives at least one potentially pharmaceutically acceptable dose of each at least two potentially pharmaceutically acceptable immunogenic agents which comprise at least one potentially pharmaceutically acceptable first pediatric immunogen and at least one agent selected from the group consisting of a second pediatric immunogen and a non-pediatric immunogen.

174 (new). The method of claim 153 wherein one screened schedule provides at least one immunogen not provided by another screened schedule or fails to provide at least one immunogen provided by another screened schedule.

175 (new). The method of claim 153 wherein one screened schedule provides a higher or lower dose of at least one immunogen than that provided for the same immunogen in said another screened schedule.

176 (new). The method of claim 153 wherein one screened schedule provides a different number of doses of at least one immunogen than is provided for the same immunogen by another screened schedule.

177 (new). The method of claim 153 wherein one screened schedule provides at least one dose of at least one immunogen at a later or earlier time from birth than the corresponding dose of the same immunogen is provided by another screened schedule.

178 (new). The method of claim 153 wherein at least one group first receives at least one immunogen starting after 41 days of life.

179 (new). The method of claim 153 wherein at least one immunogen is first administered to at least one group starting after 41 days after birth but before 180 days after birth.

180 (new). The method of claim 153 wherein at least the majority of the mammals in at least one group did not develop the infectious diseases which are associated with said immunogens.

181 (new). The method of claim 153 wherein mammals are excluded from a treatment group if:

i) said mammals have substantial immunologic protection against the infectious disease which said immunization schedule protects against, or

ii) said mammals have substantial levels of at least one surrogate marker of an autoimmune disease even though the mammals had not been previously diagnosed as having an autoimmune disease, or

iii) said surrogate marker was substantially increased following a previous vaccination, infection or other immunologic challenge.

182 (new). The method of claim 153, wherein both a pediatric immunogen and a non-pediatric immunogen are administered to at least one group.

183 (new). The method of claim 153 in which the mammals are humans and the groups are compared for a period from first administration for at least one year.

184 (new). The method of claim 153 in which the mammals are humans and the groups are compared from first administration until at least 5 years of age.

185 (new). The method of claim 153 in which the mammals are rodents and groups are compared from first administration until at least 24.5 weeks of age.

186 (new). The method of claim 153 wherein in at least one schedule at least one immunogen is administered with a depot adjuvant.

187 (new). The method of claim 153 wherein the disorder is not an immune-mediated cancer.

188 (new). The method of claim 153, further comprising determining whether the age of the subject mammal, at the time of commencement of the immunization schedule, affects the incidence, prevalence, or frequency of the disorder.

189 (new). The method of claim 153, wherein the effect of the schedules on the incidence, prevalence, or frequency of the disorder is determined at least one year after at least two of the screened immunization schedules first differ.

190 (new). The method of claim 153 in which the subject immunization schedule is identical to one of said screened schedules.

191 (new). The method of claim 153 where said subject immunization schedule also protects the subject against at least one infectious disease.

192 (new). The method of claim 153 where said subject immunization schedule also protects the subject against at least two infectious diseases.

193 (new). The method of claim 153 wherein the ability of said screened immunization schedules to protect against an infectious disease is also compared.

194 (new). The method of claim 153 wherein at least one group receives an immunogen at a time sufficiently early enough to substantially reduce the incidence of said disorder, sufficient number of mammals are followed after immunization for a sufficiently long interval to ensure that said mammals have an effect lasting for a clinically significant period of time after discontinuation of immunization, and the method does not involve the administration of an live organism leading to the infection of mammals for the duration of the time they are followed.

195 (new). The method of claim 153, wherein said chronic immune mediated disorder is diabetes mellitus, wherein the

mammals are humans, and wherein the effect of the schedules on the incidence, prevalence, frequency of the disorder is determined at least one year after said first and second immunization schedules differ.

196 (new). The method of claim 195 wherein said mammals are randomized in said groups.

197 (new). The method of claim 195 wherein both a pediatric immunogen and a non-pediatric immunogen are administered to at least one group.

198 (new). The method of claim 195 wherein at least one immunogen is administered one other than as an immunogen of a live vaccine and wherein the first dose in at least one of said immunization schedules is given when the mammals are less than 42 days old.

199 (new). The method of claim 195 wherein the incidence of the disorder in said groups is compared and wherein at least one immunogen is first administered to at least one group starting after 41 days after birth but before 180 days after birth.

200 (new). The method of claim 195, further comprising determining whether the age of the subject mammal, at the time of commencement of the immunization schedule, affects the incidence, prevalence, or frequency of the disorder, wherein at least one immunogen is administered one other than as an immunogen of a live vaccine.

201 (new). The method of claim 195 wherein incidence of the disorder in said groups is compared for at least two different chronic immune-mediated disorders, one of which is diabetes mellitus.

202 (new). The method of claim 153 where at least one screened schedule calls for immunizing mammals starting at less than 42 days after birth and the screened immunization schedules differ as to the age at the time of the first dose of at least

one other immunogen.

203 (new). The method of claim 202 where said mammals in at least one schedule receive hepatitis B immunogen prior to 42 days after birth.

204 (new). The method of claim 153 where said comparison comprises compensation for at least one confounding variable.

205 (new). The method of claim 204 where the analysis includes compensation for confounding variables selected from the group consisting of breast feeding, receiving antibiotics, the maternal age, family history of diabetes or a second chronic immune mediated disorder, maternal infections while the mammal was in utero, infections during the first 12 months of life, size of the mammal at birth, gestational age of the mammal at birth, and exposure to vaccines.

206 (new). The method of claim 153 in which, in (I)(b), the incidence, prevalence or frequency of at least one chronic immune-mediated disorder is compared, and the lower risk screened immunization schedule is associated with a lower incidence, prevalence or frequency of that disorder.

207 (new). The method of claim 153 where the first and second groups differ by at least one of the following differences:

a) the presence of at least one immunogen in the schedule for one group and not the other;
b) a difference in the size of the dose of at least one immunogen administered to both groups;
c) a difference in the number of doses of at least one immunogen administered to both groups; or
d) a difference in the day of administration, relative to birth, of the first dose of at least one immunogen administered to both groups.

208 (new). The method of claim 207 where at least one of said differences (a)-(d) relates to at least one immunogen other

than a BCG immunogen.

209 (new). The method of claim 207 where at least one of said differences (a)-(d) relates to at least one immunogen other than a BCG or measles immunogen.

210 (new). The method of claim 207 where at least one of said differences (a)-(d) relates to at least one immunogen other than a BCG, measles, mumps, rubella, smallpox, diphtheria, tetanus, pertussis or polio immunogen.

211 (new). The method of claim 207 where at least difference (a) applies.

212 (new). The method of claim 207 where at least difference (b) applies.

213 (new). The method of claim 207 where at least difference (c) applies.

214 (new). The method of claim 207 where at least difference (d) applies.

215 (new). The method of claim 207 where at least two of differences (a)-(d) apply.

216 (new). The method of claim 207 where at least three of differences (a)-(d) apply.

217 (new). The method of claim 207 where all of differences (a)-(d) apply.

218 (new). The method of claim 207 where the chronic immune-mediated disorder is diabetes.

219 (new). The method of claim 207 where the subjects are human.

220 (new). The method of claim 219 where the mammals of said groups are human.

221 (new). The method of claim 207 wherein the effect of the schedule on the incidence, prevalence, or frequency of the disorder is observed at least one year after the first difference in immunization between the groups is manifest.

222 (new). The method of claim 220 wherein the effect of

the schedule on the incidence, prevalence, or frequency of the disorder is observed at least one year after the first difference in immunization between the groups is manifest.

223 (new). A method of immunizing a mammalian subject which comprises:

(I) screening a plurality of immunization schedules, by

(a) identifying a first group of mammals and at least a second group of mammals, said mammals being of the same species, the first group of mammals having been immunized with one or more doses of one or more infectious disease-causing organism- associated immunogens according to a first screened immunization schedule, and the second group of mammals having been immunized with one or more doses of one or more infectious disease-causing organism- associated immunogens according to a second screened immunization schedule, each group of mammals having been immunized according to a different immunization schedule,
and

(b) comparing the effectiveness of said first and second screened immunization schedules in protecting against or inducing a chronic immune-mediated disorder in said first and second groups, as a result of which one of said screened immunization schedules may be identified as a lower risk screened immunization schedule and the other of said screened schedules as a higher risk screened immunization schedule with regard to the risk of developing said chronic immune

mediated disorder(s),

where the first dose of at least one infectious disease-causing organism associated immunogen given to both groups is given sooner after birth according to the first screened immunization schedule than according to the second schedule (each such immunogen so administered to said first group being hereafter referred to as an "early" immunogen regardless of its time of administration in the second group), and

(II) immunizing said subject according to a subject immunization schedule, according to which at least one of said early infectious disease-causing organism-associated immunogens is administered in accordance with said lower risk screened immunization schedule, which administration is associated with a lower risk of development of said chronic immune-mediated disorder(s) than when said immunogen was administered according to said higher risk screened immunization schedule.

224 (new). The method of claim 223 in which the disorder is an autoimmune disease.

225 (new). The method of claim 224 in which the disorder is diabetes mellitus.

226 (new). The method of claim 224 in which the disorder is SLE.

227 (new). The method of claim 224 in which at least one comparison (b) is made at least one year after first administration of an early immunogen to said mammals.

228 (new). The method of claim 224 where said mammalian subject is or said mammals are humans.

229 (new). The method of claim 225 where said mammalian subject is or said mammals are humans.

230 (new). The method of claim 229 in which at least one comparison (b) is made at least one year after first administration of an early immunogen to said mammals.

231 (new). The method of claim 230 where at least one of

said early immunogens is one other than BCG or pertussis immunogen.

232 (new). The method of claim 223 where the first dose of at least one early immunogen is given according to a screened schedule starting at less than 42 days after birth.

233 (new). The method of claim 225 where the first dose of at least one early immunogen is given according to a screened schedule starting at less than 42 days after birth.

234 (new). The method of claim 230 where the first dose of at least one early immunogen is given according to a screened schedule starting at less than 42 days after birth.

235 (new). The method of claim 231 where the first dose of at least one early immunogen is given according to a screened schedule starting at less than 42 days after birth.

236 (new). The method of claim 232 where at least two immunogens are administered according to said subject immunization schedule, and such immunogens include (1) a first immunogen which was given prior to 42 days after birth to said first and second groups, and (2) a second and different immunogen which is an early immunogen.

237 (new). The method of claim 230 further comprising (III) screening said subject, during or after receipt of said third schedule, for the development of diabetes.

238 (new). The method of claim 230 where the incidence of diabetes is compared.

239 (new). The method of claim 223 where at least two of the screened schedules also differ by either the presence of at least one immunogen or the number of doses of at least one immunogen.

240 (new). The method of claim 223 where at least one infectious disease-causing organism-associated immunogen is administered to said subject so as to protect said subject against said infectious disease.

241 (new). The method of claim 240 where at least two different immunogens are administered so as to protect the subject against at least two different infectious diseases.

242 (new). The method of claim 240 where the immunogen protective against said infectious disease is an early immunogen.

243 (new). The method of claim 223 where the screened schedules do not differ by either the presence of at least one immunogen or the number of doses of at least one immunogen.

244 (new). The method of claim 230 where said lower risk of development of diabetes is evidenced by a lower incidence or frequency, or a slower onset, of diabetes.

245 (new). A method of immunizing a mammalian subject which comprises

(I) (a) immunizing a first group of mammals with one or more doses of one or more infectious disease-causing organism-associated immunogens according to a first screened immunization schedule,

(b) immunizing at least a second group of mammals with one or more doses of one or more infectious disease-causing organism-associated immunogens according to a second screened immunization schedule, the first and second groups being of the same species, and

(c) comparing the effectiveness of said first and second screened immunization schedules in protecting against or inducing a chronic immune-mediated disorder in said first and second groups,

as a result of which one of said screened immunization schedules may be identified as a lower risk screened immunization schedule and the other of said screened schedules as a higher risk screened immunization schedule with regard to the risk of developing said chronic immune mediated disorder(s),

where the first dose of at least one infectious disease-causing organism-associated immunogen given to both groups is given sooner after birth according to the first screened immunization schedule than according to the second schedule (each such immunogen so administered to said first group being hereafter referred to as an "early" immunogen regardless of its time of administration in the second group),

and

(II) immunizing said subject according to a subject immunization schedule, according to which at least one of said early, infectious disease-causing organism-associated immunogens is administered in accordance with said lower risk screened immunization schedule, resulting in a lower risk of development of said chronic immune-mediated disorder(s) than when said immunogen was administered according to said higher risk screened immunization schedule.

246 (new). The method of claim 245 where one of said chronic immune-mediated disorders is diabetes,
where said mammalian subject is or said mammals are humans,
where said comparison (b) is made at least one year after first administration of said immunogen to said mammals.

247 (new). The method of claim 246 where at least one of said early immunogens is one other than BCG or pertussis immunogen.

248 (new). A method of protecting a mammalian subject, by immunization, against at least one infectious disease while reducing the risk of said subject thereby developing a chronic immune mediated disorder, which comprises:

(I) screening a plurality of immunization schedules, by

(a) identifying a first group of mammals and at least a second group of mammals, said mammals being of the same species, the first group of mammals having been immunized with

one or more doses of one or more infectious disease-causing organism- associated immunogens according to a first screened immunization schedule, and the second group of mammals having been immunized with one or more doses of one or more infectious disease-causing organism- associated immunogens according to a second screened immunization schedule, each group of mammals having been immunized according to a different immunization schedule,

and

(b) comparing the effectiveness of said first and second screened immunization schedules in protecting against or inducing a chronic immune-mediated disorder in said first and second groups, as a result of which one of said screened immunization schedules may be identified as a lower risk screened immunization schedule and the other of said screened schedules as a higher risk screened immunization schedule with regard to the risk of developing said chronic immune mediated disorder(s),

where the first dose of at least one infectious disease-causing organism associated immunogen given to both groups is given sooner after birth according to the first screened immunization schedule than according to the second schedule (each such immunogen so administered to said first group being hereafter referred to as an "early" immunogen regardless of its time of administration in the second group), and

(II) immunizing said subject according to a subject immunization schedule, according to which at least one of said

early infectious disease-causing organism-associated immunogens is administered in accordance with said lower risk screened immunization schedule, which administration is associated with a lower risk of development of said chronic immune-mediated disorder(s) than when said immunogen was administered according to said higher risk screened immunization schedule,

at least one of the immunogens of (II) being protective against said infectious disease when administered according to aid third immunization schedule, said third schedule presenting a reduced risk of said subject developing a chronic immune mediated disorder relative to said second schedule.

249 (new). The method of claim 248 where one of said chronic immune-mediated disorders is diabetes,
where said mammalian subject is or said mammals are humans, and where said comparison (b) is made at least one year after first administration of said immunogen to said mammals.

250 (new). A method of immunizing a mammalian subject which comprises:

(I) screening a plurality of immunization schedules, by

(a) identifying a first group of mammals and at least a second group of mammals, said mammals being of the same species, the first group of mammals having been immunized with one or more doses of one or more immunogens according to a first screened immunization schedule, and the second group of mammals having been immunized with one or more doses of one or more immunogens according to a second screened immunization schedule, each group of mammals having been immunized according to a different immunization schedule,

and

(b) comparing the incidence, frequency, prevalence, or time of onset of said chronic immune-mediated disorder in the first group with that in the second group,

where the first dose of at least one immunogen given to both groups is given (i) sooner after birth according to the first screened immunization schedule than according to the second schedule (each such immunogen so administered to said first group being hereafter referred to as an "early" immunogen regardless of its time of administration in the second group), or (ii) according to the first screened immunization schedule when the mammals of the first group are less than 42 days old (each such immunogen is administered to said first group being hereafter referred to as a "pre-42" immunogen regardless of its time of immunization in the second group);

(II) immunizing said subject according to a subject immunization schedule, according to which at least one of said early or pre-42 immunogens is administered in accordance with said first screened immunization schedule, and is associated with a lower incidence, frequency, or prevalence, or slower onset, of a chronic immune-mediated disorder than when said immunogen was administered according to said second screened immunization schedule.

251 (new). The method of claim 250 where one of said chronic immune-mediated disorders is diabetes,
where said mammalian subject is or said mammals are humans,
where said comparison (b) is made at least one year after first administration of said immunogen to said mammals.

252 (new). The method of claim 223 in which, in at least one screened schedule, the first dose of said immunization schedule is administered before the mammal's immune system arrives at a state of maturation comparable to that achieved at an age of 42 days after birth in a mouse or rat.

253 (new). The method of claim 223 in which, in said subject schedule, the first dose of said immunization schedule is administered before the subject's immune system arrives at a state of maturation comparable to that achieved at an age of 42 days after birth in a mouse or rat.

254 (new). The method of claim 225 in which, in at least one screened schedule, the first dose of said immunization schedule is administered before the mammal's immune system arrives at a state of maturation comparable to that achieved at an age of 42 days after birth in a mouse or rat.

255 (new). The method of claim 225 in which, in said subject schedule, the first dose of said immunization schedule is administered before the subject's immune system arrives at a state of maturation comparable to that achieved at an age of 42 days after birth in a mouse or rat.

256 (new). A method of protecting a mammalian subject, by immunization, against at least one infectious disease while reducing the risk of said subject thereby developing a chronic immune mediated disorder, which comprises:

immunizing said subject according to a subject immunization schedule, according to which one or more immunogens is administered to the subject, each immunogen being administered on one or more dates according to such schedule,

where it has previously been determined that the timing of first administration of at least one of said immunogens influences the risk of said subject thereby developing said disorder, and

where the first administration of at least one risk-influencing immunogen according to said schedule is timed so as to reduce the risk of said subject thereby developing said disorder, relative to the risk if said first administration had been at some later date.

257 (new). The method of claim 256 where said determination

was made by

(I) screening a plurality of immunization schedules, by

(a) identifying a first group of mammals and at least a second group of mammals, said mammals being of the same species, the first group of mammals having been immunized with one or more doses of one or more infectious disease-causing organism- associated immunogens according to a first screened immunization schedule, and the second group of mammals having been immunized with one or more doses of one or more infectious disease-causing organism- associated immunogens according to a second screened immunization schedule, each group of mammals having been immunized according to a different immunization schedule,

and

(b) comparing the effectiveness of said first and second screened immunization schedules in protecting against or inducing a chronic immune-mediated disorder in said first and second groups, as a result of which one of said screened immunization schedules may be identified as a lower risk screened immunization schedule and the other of said screened schedules as a higher risk screened immunization schedule with regard to the risk of developing said chronic immune mediated disorder(s).

258 (new). The method of claim 256 where the disorder is diabetes.

259 (new). A method of protecting a mammalian subject, by

immunization, against at least one infectious disease while reducing the risk of said subject thereby developing a chronic immune mediated disorder, which comprises:

(I) determining whether the timing of first administration of at least one immunogen protective against at least of said infectious diseases influences the risk of said subject developing said disorder, and

(II) immunizing said subject according to an immunization schedule, according to which one or more immunogens, including at least one immunogen of (I), is administered to the subject, each immunogen being administered on one or more dates according to such schedule,

where the first administration of at least one immunogen of (I) according to said schedule is timed so as to reduce the risk of said subject thereby developing said disorder, relative to the risk if said first administration had been at some later date.

Exposure to Dogs and Cats in the First Year of Life and Risk of Allergic Sensitization at 6 to 7 Years of Age

Dennis R. Ownby, MD

Christine Cole Johnson, PhD

Edward L. Peterson, PhD

Context Childhood asthma is strongly associated with allergic sensitization. Studies have suggested that animal exposure during infancy reduces subsequent allergic sensitization.

Objective To evaluate the relationship between dog and cat exposure in the first year of life and allergic sensitization at 6 to 7 years of age.

Design, Setting, and Subjects Prospective birth cohort study of healthy, full-term infants enrolled in a health maintenance organization in suburban Detroit, Mich, who were born between April 15, 1987, and August 31, 1989, and followed up yearly to a mean age of 6.7 years. Of 835 children initially in the study at birth, 474 (57%) completed follow-up evaluations at age 6 to 7 years.

Main Outcome Measures Atopy, defined as any skin prick test positivity to 6 common aeroallergens (dust mites [*Dermatophagoides farinae, D pteronyssinus*], dog, cat, short ragweed [*Ambrosia artemisiifolia*], and blue grass [*Poa pratensis*]); seroatopy, defined as any positive allergen-specific IgE test result for the same 6 allergens or for *Alternaria* species.

Results The prevalence of any skin prick test positivity (atopy) at age 6 to 7 years was 33.6% with no dog or cat exposure in the first year of life, 34.3% with exposure to 1 dog or cat, and 15.4% with exposure to 2 or more dogs or cats ($P=.005$). The prevalence of any positive allergen-specific IgE test result (seroatopy) was 38.5% with no dog or cat exposure, 41.2% with exposure to 1 dog or cat, and 17.9% with exposure to 2 or more dogs or cats ($P=.003$). After adjustment for cord serum IgE concentration, sex, older siblings, parental smoking, parental asthma, bedroom dust mite allergen levels at 2 years, and current dog and cat ownership, exposure to 2 or more dogs or cats in the first year of life was associated with a significantly lower risk of atopy (adjusted odds ratio, 0.23; 95% confidence interval, 0.09-0.60) and seroatopy (adjusted odds ratio, 0.33; 95% confidence interval, 0.13-0.83).

Conclusion Exposure to 2 or more dogs or cats in the first year of life may reduce subsequent risk of allergic sensitization to multiple allergens during childhood.

JAMA. 2002;288:963-972

www.jama.com

THE INCREASING PREVALENCE OF asthma in the United States and other developed countries over the last few decades has been a cause for concern.[1,2] While many factors appear to be involved in the development of childhood asthma, allergic sensitization to common allergens has consistently been shown to be related to the risk of developing asthma and to the risk of asthma persisting from childhood into adulthood.[3-6] Many studies have attempted to elucidate relationships between environmental exposures, especially during infancy, and the risk of allergic sensitization in later life.[7,8] These studies are based on the theory that an individual's genetic predisposition to allergic disease is activated or enhanced by early allergen exposure.[4,7,9] The outcome of interactions between genetic influences and allergen exposures may be influenced by other environmental exposures, such as passive exposure to environmental tobacco smoke.[7,9] If these relationships were better understood it might become possible to reduce the prevalence of allergic sensitization and perhaps asthma in children.

Exposure to dogs and cats during infancy has been thought to increase the

For editorial comment see p 1012.

Author Affiliations: Section of Allergy and Immunology, Department of Pediatrics, Medical College of Georgia, Augusta (Dr Ownby); Department of Biostatistics and Research Epidemiology, Henry Ford Health System, Detroit, Mich (Drs Johnson and Peterson); National Institute of Environmental Health Sciences Center in Molecular and Cellular Toxicology with Human Applications, Wayne State University, Detroit (Drs Johnson and Peterson).

Financial Disclosure: Dr Ownby has received honoraria from AstraZeneca, Genentech, GlaxoSmithKline, Schering, Merck, and Aventis.

Corresponding Author and Reprints: Dennis R. Ownby, MD, Section of Allergy and Immunology, Department of Pediatrics, BG-1019, Medical College of Georgia, Augusta, GA 30912-3790 (e-mail: downby@mail.mcg.edu).

risk of subsequent allergy to these animals.[8,10-12] This assumption is primarily based on a few retrospective studies reporting an increased likelihood of allergic sensitization following exposure during infancy.[10-12] Some studies, however, have suggested that exposure to dogs or cats during infancy is associated with reduced risk of allergic disease.[13-18] Others have shown that children growing up on farms, especially farms with animals, were less likely to be allergic than were children growing up in urban environments.[19,20]

This analysis is part of the Childhood Allergy Study, a prospective birth cohort study designed to simultaneously evaluate multiple relationships between early environmental exposures and subsequent allergic sensitization and asthma.[21-30] Among the variables considered were parental allergy histories, parental smoking, IgE levels in cord blood, month of birth, concentrations of dust mite and cat allergen in the child's bedroom at age 2 years, and pet exposure. In this analysis, we specifically examined exposure to dogs or cats in the first year of life and a child's risk of later allergic sensitization to common allergens after adjusting for potential confounding associations. We also examined relationships between early dog and cat exposure and allergen-specific serum IgE concentrations, lung function, methacholine airway responsiveness, and asthma.

METHODS

The selection of children for the Childhood Allergy Study has previously been described.[21] Briefly, all pregnant women living in an area of northern, suburban Detroit, defined by contiguous ZIP codes, and belonging to a health maintenance organization, were eligible to participate if their infants were born between April 15, 1987, and August 31, 1989. Only infants born at term (36 or more weeks' gestational age) with valid measurements of cord serum IgE concentration were entered into the study. The study was approved by the institutional human rights committee, and written informed consent was obtained when the mothers were enrolled, at the time of the first home visit, and prior to the clinical evaluations.

Study nurses interviewed mothers prior to delivery to obtain information concerning each parent's level of education; presence of allergies in general and of hay fever and asthma specifically; and parental smoking habits. The number of siblings was also noted along with other data about the home. Cord serum IgE concentrations were measured for all infants as previously described.[21]

We contacted parents by telephone when infants were aged 1 year to obtain information on prespecified variables of interest, including the presence and number of pets in the home during the first year. The number of dogs and cats reported at this time was used for this analysis. When children were aged 2 years, nurses visited each child's home to obtain information about the home environment and to collect dust samples from the child's bedroom, as well as urine samples from the child for measurement of urinary cotinine as a biomarker of passive cigarette smoke exposure. The dust samples were analyzed for concentrations of mite (Der p 1 and Der f 1) and cat (Fel d 1) allergens using a monoclonal antibody-based enzyme-linked immunosorbent assay as previously described.[28] We have documented the validity of parental smoking histories in this cohort with reference to children's urinary cotinine concentrations.[26] Questionnaire-based parental smoking histories from the first year of the child's life were used for these analyses because there were fewer missing values than for urinary cotinine concentrations. Follow-up telephone interviews also were conducted when the children were aged 3, 5, and 6 years, and a second home visit was conducted when the children were aged 4 years.

Evaluations for Allergic Sensitization and Asthma

Clinical evaluations for allergic sensitization and asthma were performed when the children were aged 6 to 7 years. In addition to general medical histories and physical examinations, these evaluations included skin prick testing with commercial extracts of dust mites (*Dermatophagoides farinae*, *D pteronyssinus*), dog, cat, short ragweed (*Ambrosia artemisiifolia*), and blue grass (*Poa pratensis*), along with saline and histamine controls (all extracts and controls, Pharmaceutical Division, Bayer Inc, Spokane, Wash). Skin prick test results were considered positive if the product of perpendicular wheal diameters was 4 mm or more associated with a flare of at least 10 mm, and if there was no response to the negative control. Atopy was defined as a positive skin prick test result with any of the 6 allergens tested. Blood samples obtained during the evaluation were assayed for total serum IgE concentration and concentrations of allergen-specific IgE antibodies using a commercial assay (AlaSTAT, Diagnostic Products Corp, Los Angeles, Calif). Allergen-specific IgE testing included the same 6 allergens used for skin prick testing in addition to *Alternaria* species. Total and allergen-specific serum IgE levels were expressed in international units per milliliter (1 IU/mL corresponds to 2.4 ng/mL). Allergen-specific IgE levels of 0.35 IU/mL or higher were considered to be a positive test result in accordance with the manufacturer's recommendation. Seroatopy was defined as any positive test result for an allergen-specific IgE concentration. Numbers of children with seroatopy may have been slightly higher than those with atopy because one additional allergen, *Alternaria*, was also used to define seroatopy. Because a study published after the start of this study suggested that cockroach sensitization may be associated with asthma,[31] a random sample of 100 sera were assayed for cockroach-specific IgE and only 2 sera were positive.[24] Given the low prevalence of detectable cockroach sensitization, no further testing for cockroach-specific IgE was performed.

At the time of skin prick testing, children were defined as having current asthma if a parent reported that they had been diagnosed by a physician as having asthma and that they had asthma

symptoms or used asthma medications in the preceding 12 months. Pulmonary function tests were performed as previously described, and the results are presented as the percentage of predicted using standard equations.[29] Methacholine airway responsiveness was determined as previously described.[29] After baseline spirometry and no response to a control saline challenge, 5 doses of methacholine (0.025-25 mg/mL) were administered through a dosimeter (Pulmonary Data Services, Louisville, Colo). Methacholine airway responsiveness was defined as a fall in forced expiratory volume in 1 second (FEV_1) of 20% or more from the postsaline challenge value at a concentration of administered methacholine of 10 mg/mL or less.[29]

Statistical Analysis

The power of this study was originally based on the ability to detect a small to medium effect (0.2) for a χ^2 test as defined by Cohen.[32] With this assumption, and assuming an α level of .05, the power to detect significant associations between outcomes in 3 exposure groups is greater than 90% with an overall sample size of 470. Given the same assumptions, it also is possible to stratify the data for a variable with approximately equal prevalences (ie, sex) and still have power greater than 70% with 235 in each group. If the prevalence of a variable is low in the cohort, such as current asthma, the power is much lower.

The collected data were first examined for potential imbalances between those children lost from the study and those who were retained. χ^2 Tests were used to compare the relative percentages.[33]

Pet exposure in the first year of life was defined as an ordinal variable with 3 categories: no dog or cat exposure, exposure to 1 dog or cat, and exposure to 2 or more dogs or cats. The highest strata was truncated at 2 or more because of the small sample size above this level. Information about pet exposure at age 6 to 7 years was used to create the same 3 categories of pet exposure as was used for the first year of life. Binary variables of interest (eg, atopy [yes or no], specific skin prick test positivity) were analyzed according to pet exposure category using a χ^2 test for 2×3 contingency tables.[33] We did not have a preconceived hypothesis concerning a relationship between exposure to varying number of dogs and cats and the risk of allergic sensitization. Therefore, we tested the general hypothesis that outcomes differed across categories of dog and cat exposure rather than testing for trends with increasing exposure.

The relationships between pet exposure categories and continuous variables, such as percent predicted forced vital capacity (FVC) or total serum IgE, were evaluated with a 1-way analysis of variance technique.[34] Each continuous variable was transformed to natural logarithmic equivalents to reduce positive skewing prior to analysis. If the range of the variable to be logarithmically transformed included zero, 1 was added to the variable prior to transformation. When logarithmic data transformation did not result in a near normal distribution, such as for dust mite concentrations, a Kruskal-Wallis test was used. The number of children in each analysis varied slightly because of missing data.

Atopy and seroatopy were each used as a dependent binary variable in a linear logistic regression assessing 2 indicator variables for pet exposure: exposure to 1 dog or cat or exposure to 2 or more dogs or cats.[35] These models were fitted without other variables and with other potentially confounding variables, including cord serum IgE concentration, child's sex, having older siblings, parental smoking, mother or father with a history of asthma, and total bedroom dust mite allergen levels at child age 2 years. The logistic model is appropriate for modeling binary dependent variables. It makes minimal assumptions about the distributional properties of the independent variables and the exponentiation of the coefficient allows for estimation of the odds ratio (OR). We chose to include the number of dogs and cats as 2 binary indicator variables avoiding assumptions concerning the direction of any associations. Using the Hosmer-Lemeshow test, we found no evidence to doubt the validity of the models.[35]

We analyzed the entire data set and data sets defined by sex. In all analyses, an $\alpha = .05$ criteria was used to determine statistical significance. There was no attempt to impute data; all analyses were performed on all available data. SAS v8.0 (SAS Institute Inc, Cary, NC) was used for all analyses.[36]

RESULTS

A total of 1194 pregnant women were potentially eligible for entry into this study, and consent for participation was obtained from 953 women. Infants of 106 of these women were not enrolled in the study because a cord blood sample was not obtained, leaving 847 eligible newborns. Six of the cord blood samples were thought to be contaminated by maternal blood,[21,23] and an additional 6 children were found to be ineligible when each child's data were examined and verified prior to the 6- to 7-year evaluations, yielding 835 eligible children enrolled at birth. Of the 835 children initially enrolled, 235 had been lost to follow-up by age 6 years, and 126 of those contacted at age 6 years declined participation in the clinical evaluation. Thus, 474 (57%) of the 835 eligible children initially enrolled completed the clinical evaluation for allergic sensitization and asthma at an average age of 6.7 (SD, 0.17) years. Characteristics of children who were evaluated at 6 to 7 years did not differ significantly from those of children who did not undergo clinical evaluation at age 6 to 7 years, including whether the parent had a history of asthma or hay fever or whether there were dogs or cats in the household (TABLE 1). Also, interactions between each variable, any exposure to dogs and cats in the home in the first year of life, and whether the child participated in the clinical evaluation were not statistically significant (Table 1). When the relationship between maternal and paternal histories of asthma, allergies, and hay fever and presence of 2 or more dogs or cats in

the household was evaluated, no significant associations were found.

The parents of the children in this study were relatively well educated and almost all (804 [96.3%]) described themselves as white, non-Hispanic. Characteristics of the children completing the study are presented in TABLE 2. Boys and girls were approximately equally represented. The presence of a dog or cat in the home did not differ significantly between parents with a history of asthma, allergies, or hay fever and those who did not report these conditions.

To investigate the relationships between dog and cat exposure and allergic sensitization, we initially compared the 184 children with any dog exposure in their first year of life to the 220 children without either dog or cat exposure. Children exposed to a dog were less likely to have a positive skin test result to dog allergen (3.3% vs 8.6%, $P=.03$) and detectable dog-specific IgE (3.7% vs 8.7%, $P=.06$) at follow-up. Any exposure to a dog was also associated with lower total serum IgE levels (geometric mean, 23.8 IU/mL vs 33.1 IU/mL for no dog or cat exposure; $P=.04$). The inverse association between dog exposure and allergic sensitization was further examined in relationship to number of dogs (TABLE 3).

Table 1. Comparison of Children in the Cohort Who Underwent Clinical Evaluation at Age 6 to 7 Years With Those Not Evaluated*

Variable	No./Total (%) Not Evaluated	No./Total (%) Evaluated	P Value Evaluated vs Not Evaluated	P Value For Cat and Dog Interaction†
Children				
Female	178/351 (50.7)	247/484 (51.0)	.93	.71
First-born	148/321 (46.1)	214/478 (44.8)	.71	.13
Exposure to dogs and cats in first year of life				
Dogs or cats				
0	139/285 (48.8)	223/474 (47.1)	.20	NA
1	88/285 (30.9)	173/474 (36.5)		
≥2	58/285 (20.4)	78/474 (16.5)		
Dogs only	113/252 (44.8)	184/407 (45.2)	.93	NA
Cats only	70/209 (33.5)	106/329 (32.2)	.76	NA
Parental Characteristics				
Smoking ≥1 cigarette per day				
Mother	66/351 (18.8)	78/484 (16.1)	.31	.46
Father	85/348 (24.4)	116/482 (24.1)	.90	.82
Asthma				
Mother	24/351 (6.8)	41/483 (8.5)	.38	.87
Father	28/333 (8.4)	25/463 (5.4)	.09	.46
Either parent	47/335 (14.0)	64/463 (13.8)	.93	.42
Allergy				
Mother	99/351 (28.2)	141/484 (29.1)	.77	.18
Father	86/321 (26.8)	113/429 (26.3)	.89	.69
Either parent	159/333 (47.8)	219/447 (49.0)	.73	.18
Hay fever				
Mother	62/350 (17.7)	76/483 (15.7)	.45	.09
Father	62/320 (19.4)	88/445 (19.8)	.89	.68
Either parent	108/329 (32.8)	142/453 (31.4)	.66	.83
Formal education beyond high school‡				
Mother	219/351 (62.4)	299/484 (61.8)	.86	.25
Father	239/349 (68.5)	331/483 (68.5)	.99	.40

*NA indicates not applicable.
†For interaction between any exposure to cats and dogs in the first year of life, the variable of interest, and being evaluated at age 6 to 7 years.
‡Includes some college or technical school training.

The reference group remained the 220 children with neither dog nor cat exposure in the first year of life. An apparent dose-response effect for atopy and seroatopy was found across the 3 exposure categories. Atopy was present in 33.6% of the children without dog or cat exposure, in 29.7% with exposure to 1 dog, and in only 8.3% with exposure to 2 or more dogs ($P=.009$). The prevalence of seroatopy was 38.5% with no pet exposure, 36.7% with exposure to 1 dog, and 12.9% with exposure to 2 or more dogs ($P=.02$). The same analyses were performed with cat exposure during the first year. Using the same reference group of 220 unexposed children, the patterns toward less prevalent allergic sensitization with exposure to cats were similar to those observed with dog exposure, but none of the associations reached statistical significance. For example, the prevalence of atopy declined from 33.6% to 31.4% to 23.1% with exposure to no dogs or cats, 1 cat, or 2 or more cats, respectively ($P=.54$ for comparison across categories), while seroatopy declined from 38.5% to 34.5% to 26.1% ($P=.45$).

Based on finding similar relationships between dog and cat exposures in the first year of life and allergic sensitization at age 6 to 7 years, relationships were further analyzed by simultaneously considering combined dog and cat exposure. Combining dogs and cats increased the number of children in each category, allowing further exploration of the relationships through stratification of the data by sex. Atopy and seroatopy were each present in about one third of children. When all children were considered, the prevalence of skin prick test positivity to dog allergen, outdoor and indoor allergens, atopy, and seroatopy were significantly different across the 3 exposure categories and generally decreased with increasing pet exposure (TABLE 4). The pattern of decreasing skin prick test positivity to cat allergen with increasing exposure was similar but the relationship(s) failed to reach statistical significance.

Table 2. Characteristics of 474 Children Evaluated for Allergic Sensitization and Asthma

Characteristic	All Subjects	Dog or Cat Exposure in First Year of Life		
		0 Dogs or Cats	1 Dog or Cat	≥2 Dogs or Cats
Age, mean (SD) [range], y	6.72 (0.17) [6.1-7.7]	6.71 (0.16)	6.74 (0.18)	6.69 (0.17)
Cord serum IgE, mean (SD) [range], IU/mL*	0.28 (0.27) [0.01-2.13]	0.28 (0.28)	0.27 (0.24)	0.32 (0.28)
Dust mite concentration (Der f 1 + Der p 1) in child's room at age 2 years, mean (SD) [range], μg/g dust†	0.82 (1.33) [−0.68 to 5.85]	0.93 (1.37)	0.72 (1.22)	0.67 (1.42)
Girls:boys (percentage)	242:232 (51:49)	113:110 (51:49)	90:83 (52:48)	39:39 (50:50)
No siblings, No./total (%)	210/469 (44.8)	92/221 (41.6)	78/173 (45.1)	40/75 (53.3)
Either parent smokes, No./total (%)	143/472 (30.3)	57/223 (25.6)	59/172 (34.3)	24/77 (35.1)
Dog and cat exposure in first year of life, No./total (%)				
Dog	184/474 (38.8)		125/173 (72.3)	59/78 (75.6)
Cat	106/474 (22.4)		49/173 (28.3)	57/78 (73.1)
Dog or cat	251/474 (53.0)			
Any dog or cat exposure by parental history, No./total (%)‡				
Parental asthma				
Present	56/100 (56.0)	$P = .49$		
Absent	195/374 (52.1)			
Parental allergy				
Present	116/216 (53.7)	$P = .41$		
Absent	110/221 (50.2)			
Parental hay fever				
Present	81/141 (57.4)	$P = .17$		
Absent	153/303 (50.5)			

*Values represent the natural logarithm of the IgE concentration plus 1.
†Values represent the natural logarithm of dust mite concentrations.
‡Dog or cat in the home in the first year of the child's life when either parent has or does not have a history of asthma, allergies, or hay fever.

When boys and girls were considered separately, different patterns emerged from the data (Table 4). Exposure to a single dog or cat in the first year of life was associated with an increased prevalence of atopy and seroatopy in girls while both outcomes declined in boys exposed to a single dog or cat. Lower prevalences of skin prick test positivity to dog, cat, and indoor and outdoor allergens, and of methacholine airway responsiveness were consistently found in association with exposure to a single dog or cat with boys but not with girls. Measurements of lung function were also related to dog and cat exposure for boys but not for girls. The prevalence of methacholine airway responsiveness in boys was 25.5% when there had been no dog or cat exposure, 20.3% with exposure to 1 dog or cat, and 5.1% with exposure to 2 or more dogs or cats ($P = .03$). In girls, the prevalence of methacholine responsiveness was unchanged across pet exposure categories. Similarly, the mean percent predicted FVC and FEV_1 increased significantly across pet exposure categories among boys but not among girls, and were highest with exposure to 2 or more dogs or cats. Thirty-three (7%) of 473 children had current asthma. The prevalence of current asthma was lower in boys who had been exposed to 2 or more dogs or cats in infancy compared with no exposure (5.1% vs 11.8%, respectively), but the difference across exposure categories was not statistically significant ($P = .43$) and no difference was seen for girls.

Table 3. Relationship Between the Number of Dogs in the Home in the First Year of Life and the Prevalence of Allergic Sensitization at Age 6 to 7 Years*

Variable	No./Total (%)			P Value†
	No Dog or Cat Exposure	No. of Dogs: 1	No. of Dogs: ≥2	
Skin prick test positivity				
Dog	19/220 (8.6)	6/148 (4.1)	0/36 (0)	.06
Cat	34/220 (15.5)	16/148 (10.8)	1/36 (2.8)	.07
Outdoor allergens‡	62/206 (30.1)	28/137 (20.4)	2/27 (7.4)	.01
Indoor allergens§	60/220 (27.3)	37/148 (25.0)	2/36 (5.6)	.02
Atopy‖	74/220 (33.6)	44/148 (29.7)	3/36 (8.3)	.009
Seroatopy¶	74/192 (38.5)	47/128 (36.7)	4/31 (12.9)	.02
Methacholine airway responsiveness	53/220 (24.1)	34/144 (23.6)	4/34 (11.8)	.27
Current asthma	17/223 (7.6)	9/147 (6.1)	2/36 (5.6)	.81

*The group not exposed to dogs was further restricted to those children without any cat exposure in the first year of life since both dog and cat exposure appeared to be associated with the variables presented in this table.
†From χ^2 analysis comparing each of the 3 categories of dog exposure.
‡Short ragweed (*Ambrosia artemisiifolia*), blue grass (*Poa pratensis*), and *Alternaria*.
§*Dermatophagoides farinae*, *D pteronyssinus*, dog, and cat.
‖Positive skin test to 1 or more of *D farinae*, *D pteronyssinus*, dog, cat, short ragweed, or blue grass.
¶Positive in vitro test for IgE specific to *D farinae*, *D pteronyssinus*, dog, cat, short ragweed, blue grass, or *Alternaria*.

For all children, exposure to 2 or more dogs and cats in the first year of life was associated with a lower total serum IgE at age 6 to 7 years, but the analysis across exposure categories was not statistically significant (P=.09) (TABLE 5). In analyses stratified by sex, exposure to more dogs or cats was associated with significantly decreased geometric mean IgE among boys (P=.02) and among children with a parental history of asthma (P=.03), but not among girls (P=.42) or among children without a parental history of asthma (P=.31).

When mean total dust mite concentrations in the child's bedroom at age 2 years were compared for homes with no pet, 1 dog or cat, and 2 or more dogs or cats, the median (5th percentile, 95th percentile) dust mite concentrations were not significantly different (2.0 [0.5, 37.1] µg/g dust; 1.8 [0.5, 16.6] µg/g dust; 1.1 [0.5, 43.8] µg/g dust; P=.27).

Logistic regression analysis was used to adjust for the effects of possible confounding variables (cord serum IgE concentration, levels of house dust mite allergen in the child's bedroom at age 2 years, child's sex, an older sibling, passive exposure to parental tobacco smoke, and parental history of asthma) on relationships between dog and cat exposure and risks of atopy and seroatopy. After adjusting for all of these variables, exposure to 2 or more dogs or cats was still associated with significantly lower risks of atopy (OR, 0.31; 95% confidence interval [CI], 0.14-0.72) and seroatopy (OR, 0.43; 95% CI, 0.19-0.96) in all children (TABLE 6, model 1).

When the analysis was further adjusted for current exposure to dogs or

Table 4. Relationship Between Number of Dogs and Cats in the Home in the First Year of Life, Prevalence of Allergic Sensitization, and Measures of Lung Function or Presence of Current Asthma at Age 6 to 7 Years*

Variable	Sex	No. of Dogs and Cats: 0	1	≥2	P Value†
Skin prick test positivity					
Dog	All	19/220 (8.6)	6/172 (3.5)	2/78 (2.6)	.04
	Girls	6/112 (5.4)	4/90 (4.4)	1/39 (2.6)	.77
	Boys	13/108 (12.0)	2/82 (2.4)	1/39 (2.6)	.02
Cat	All	34/220 (15.5)	20/172 (11.6)	6/78 (7.7)	.18
	Girls	12/112 (10.7)	13/90 (14.4)	2/39 (5.1)	.30
	Boys	22/108 (20.4)	7/82 (8.5)	4/39 (10.3)	.05
Outdoor allergens‡	All	62/206 (30.1)	37/160 (23.1)	8/66 (12.1)	.01
	Girls	25/104 (24.0)	20/84 (23.8)	2/32 (6.3)	.08
	Boys	37/102 (36.3)	17/76 (22.4)	6/34 (17.7)	.04
Indoor allergens‡	All	60/220 (27.3)	49/172 (28.5)	8/78 (10.3)	.005
	Girls	23/112 (20.5)	28/90 (31.1)	2/39 (5.1)	.004
	Boys	37/108 (34.3)	21/82 (25.6)	6/39 (15.4)	.07
Atopy‡	All	74/220 (33.6)	59/172 (34.3)	12/78 (15.4)	.005
	Girls	30/112 (26.8)	34/90 (37.8)	3/39 (7.5)	.002
	Boys	44/108 (40.7)	25/82 (30.5)	9/39 (23.1)	.10
Seroatopy‡	All	74/192 (38.5)	61/148 (41.2)	12/67 (17.9)	.003
	Girls	28/93 (30.1)	32/76 (42.1)	6/37 (16.2)	.02
	Boys	46/99 (46.5)	29/72 (40.3)	6/30 (20.0)	.04
Methacholine airway responsiveness§	All	53/220 (24.1)	40/166 (24.1)	12/76 (15.8)	.29
	Girls	25/110 (22.7)	24/87 (27.6)	10/37 (27.0)	.71
	Boys	28/110 (25.5)	16/79 (20.3)	2/39 (5.1)	.03
FVC % predicted, mean (SD) [No. of children]¶	All	93.9 (11.7) [222]	96.5 (11.4) [169]	95.1 (12.4) [77]	.10‖
	Girls	94.4 (11.4) [112]	95.4 (10.7) [88]	91.0 (12.7) [38]	.14‖
	Boys	93.5 (12.1) [110]	97.7 (12.1) [81]	99.0 (10.9) [39]	.01‖
FEV_1 % predicted, mean (SD) [No. of children]¶	All	93.1 (11.6) [222]	94.5 (12.2) [169]	94.7 (12.6) [77]	.41‖
	Girls	92.3 (11.0) [112]	91.8 (11.0) [88]	89.1 (12.0) [38]	.29‖
	Boys	93.8 (12.2) [110]	97.4 (12.8) [81]	100.1 (10.7) [39]	.01‖
Current asthma#	All	17/223 (7.6)	12/172 (7.0)	4/78 (5.1)	.76
	Girls	4/113 (3.5)	5/89 (5.6)	2/39 (5.1)	.77
	Boys	13/110 (11.8)	7/83 (8.4)	2/39 (5.1)	.43

*All data are No./Total (%) unless indicated otherwise. FVC indicates forced vital capacity; FEV_1, forced expiratory volume in 1 second.
†From χ^2 analysis except where noted.
‡See Table 3 footnote for definitions of outdoor and indoor allergens, atopy, and seroatopy.
§Defined as a provocative dose of ≤10 mg/mL for decreasing FEV_1 by 20%.
‖From analysis of variance.
¶Results are presented as mean (SD) of the percent predicted from standard equations (see Ownby et al[23] for details).
#Defined as physician diagnosis of asthma and symptoms of asthma or use of asthma medications in the previous 12 months.

cats using the same exposure categories as in the first year of life (no exposure; exposure to 1 dog or cat; exposure to 2 or more dogs or cats at age 6-7 years), the risk of atopy and seroatopy associated with exposure in the first year of life remained significantly decreased (atopy: OR, 0.23; 95% CI, 0.09-0.60; seroatopy: OR, 0.33; 95% CI, 0.13-0.83) (Table 6, model 2). When the variable for dog or cat exposure at age 6 to 7 years replaced the variable for exposure in the first year of life, no statistically significant associations were found. For example, with all children included in the analysis, risks of atopy and seroatopy among children with 2 or more dogs or cats at age 6 to 7 years were not significantly different than those among children with no current pet exposure (for atopy: OR, 0.79; 95% CI, 0.44-1.85; $P=.79$; for seroatopy: OR, 0.81; 95% CI, 0.43-1.94; $P=.81$).

COMMENT

In this prospective study we found that exposure to 2 or more dogs or cats in the first year of life was associated with a lower prevalence of allergic sensitization at age 6 to 7 years regardless of exposure to dogs and cats at age 6 years. This inverse relationship was consistent whether skin prick tests for 6 common aeroallergens or tests for 7 allergen-specific IgE concentrations were considered as primary outcomes. The inverse relationship was present for both indoor (dust mites, dog, and cat) and outdoor (ragweed, grass, and *Alternaria*) allergens. The relationships remained significant after adjusting for a number of variables that may be risk

Table 5. Relationship Between Number of Dogs and Cats in the Home in the Child's First Year of Life and Total Serum IgE Concentration at Age 6 to 7 Years*

	No Dogs or Cats			1 Dog or Cat			≥2 Dogs or Cats			
	No.	Mean†	Ln Mean (SD)‡	No.	Mean†	Ln Mean (SD)‡	No.	Mean†	Ln Mean (SD)‡	*P* Value§
All children	204	32.8	3.49 (1.47)	155	26.8	3.29 (1.46)	72	21.8	3.08 (1.21)	.09
Girls	100	24.0	3.18 (1.24)	81	28.5	3.35 (1.43)	37	20.5	3.02 (1.18)	.42
Boys	104	43.8	3.78 (1.61)	74	25.0	3.22 (1.50)	35	23.1	3.14 (1.26)	.02
Child's parents free of asthma	163	29.4	3.38 (1.41)	126	23.6	3.16 (1.48)	50	22.7	3.12 (1.35)	.31
Child's parent has asthma	41	48.9	3.89 (1.63)	29	46.5	3.84 (1.22)	22	19.7	2.98 (0.083)	.03

*Ln indicates natural logarithm.
†Geometric mean of the total serum IgE for each group, in IU/mL.
‡Natural logarithm of the mean serum IgE and SD of the Ln mean.
§*P* value from analysis of variance comparing the 3 Ln means.

Table 6. Adjusted Relationships Between Dog and Cat Exposure, Atopy, and Seroatopy*

Variable	OR (95% CI)	*P* Value	Model 1† Adjusted OR (95% CI)	Model 1† *P* Value	Model 2‡ Adjusted OR (95% CI)	Model 2‡ *P* Value
Exposure to 1 Dog or Cat in First Year of Life						
All children						
Atopy	1.03 (0.68-1.57)	.89	1.01 (0.61-1.67)	.97	0.85 (0.48-1.52)	.58
Seroatopy	1.12 (0.72-1.73)	.62	1.20 (0.71-2.02)	.50	0.93 (0.51-1.68)	.80
Girls						
Atopy	1.66 (0.91-3.02)	.10	1.99 (0.95-4.17)	.07	2.02 (0.84-4.84)	.11
Seroatopy	1.69 (0.89-3.19)	.11	2.21 (1.03-4.72)	.04	1.78 (0.73-4.34)	.20
Boys						
Atopy	0.64 (0.35-1.17)	.15	0.54 (0.25-1.15)	.11	0.36 (0.15-0.87)	.02
Seroatopy	0.78 (0.42-1.44)	.42	0.63 (0.29-1.37)	.25	0.46 (0.19-1.12)	.09
Exposure to ≥2 Dogs or Cats in First Year of Life						
All children						
Atopy	0.36 (0.18-0.71)	.003	0.31 (0.14-0.72)	.007	0.23 (0.09-0.60)	.003
Seroatopy	0.35 (0.18-0.69)	.003	0.43 (0.19-0.96)	.04	0.33 (0.13-0.83)	.02
Girls						
Atopy	0.23 (0.07-0.80)	.02	0.21 (0.04-1.00)	.05	0.15 (0.02-0.90)	.04
Seroatopy	0.45 (0.17-1.20)	.11	0.62 (0.20-1.96)	.42	0.54 (0.14-2.08)	.37
Boys						
Atopy	0.44 (0.19-1.01)	.05	0.37 (0.13-1.05)	.06	0.23 (0.07-0.79)	.02
Seroatopy	0.29 (0.11-0.77)	.01	0.30 (0.09-0.96)	.04	0.21 (0.06-0.79)	.02

*OR indicates odds ratio; CI, confidence interval.
†Odds ratio and 95% CI with reference to no dog or cat exposure in the first year of life adjusted for cord serum IgE concentration, total dust mite allergen (Der f 1+ Der p 1) in child's bedroom at age 2 years, child's sex, older siblings, parental smoking, and parental history of asthma.
‡Odds ratio and 95% CI adjusted for variables in model 1 plus dog and cat exposure at age 6 to 7 years.

factors for allergic sensitization or that could have been associated with pet ownership, including cord serum IgE concentration, house dust mite exposure, older siblings, parental smoking, and parental history of asthma.[4,37-42]

Other studies have also reported lower prevalences of allergic sensitization or symptoms related to allergic diseases in association with early exposure to dogs and cats,[13,15,16,43,44] but a systematic review of the literature concerning this question concluded that previous exposure to dogs and cats increased the risk of asthma and wheezing in children older than 6 years.[45] The conclusions of this systematic review differ from the results of 2 large prospective birth cohort studies.[43,44] Nafstad et al[43] found that after using logistic regression to adjust for potential confounders, being exposed to pets in early life reduced the risk of asthma (OR, 0.7; 95% CI, 0.5-1.1) and allergic rhinitis (OR, 0.6; 95% CI, 0.4-1.0) in a birth cohort of 2531 children followed to age 4 years. In a birth cohort of 1246 children in Arizona followed up to age 13 years, Remes et al[44] reported that children who had 1 or more dogs in the home at birth were significantly less likely to develop frequent wheeze than children without early dog exposure, but neither early exposure to dogs or to cats was associated with skin prick test positivity or total serum IgE concentrations. Remes et al did not find a difference between children exposed to 1 dog compared with those exposed to 2 or more dogs. They also found that the inverse relationship between dog exposure and frequent wheeze was predominantly among children without a parental history of asthma. Reasons for the differences in allergic sensitization outcomes between our study and the study by Remes et al are not clear, but may include differences in climate where the birth cohorts were located and differences in keeping pets inside the home.

In a recent cross-sectional study in children by Platts-Mills et al,[18] and also in a subsequent study in adults,[46] a bell-shaped dose-response relationship between cat allergen exposure and cat-specific sensitization was observed. Decreased levels of cat-specific sensitization were associated with both the lowest and the highest cat allergen exposure groups. Platts-Mills and colleagues also found that cat-specific IgG antibody levels increased with increasing cat exposure, and were highest in the highest cat exposure group. They suggested that high levels of cat allergen exposure induced a modified T helper cell type 2 (T_H2) response with production of cat allergen–specific IgG and IgG4 antibodies without allergic sensitization. This interesting hypothesis is not entirely consistent with the data presented in our study because we found that allergen-specific IgE antibodies to dust mites, ragweed, and grass (allergens unrelated to dog and cat) were also less prevalent in children exposed to dogs and cats in the first year of life.

Other researchers have suggested that the protective effect of dogs and cats is not related to allergen exposure but rather to increased exposure to bacterial endotoxin associated with household pets.[15,47] Endotoxin exposure is hypothesized to shift the developing immune system away from a T_H2-type pattern of response, which favors development of allergic sensitization, toward a T_H1-type response. Studies in animals have shown that concomitant exposure to endotoxin and allergen will prevent allergic sensitization normally induced by the allergen.[48] Recent studies have shown that endotoxin levels in homes are inversely related to T_H2-type cytokine production by lymphocytes of children residing in the homes and that the presence of household dogs is related to higher levels of indoor endotoxin.[47,49] Our data are consistent with the hypothesis that exposure to 2 or more dogs or cats, and therefore exposure to higher levels of endotoxin, is associated with a T_H1 pattern of immune response and less allergic sensitization.

There were several associations with dog and cat exposure that were evident for boys but not for girls, including lower total serum IgE concentrations, lower prevalence of methacholine airway responsiveness, and better lung function. These differences in associations between boys and girls are puzzling, but others have also observed differences between boys and girls in factors related to asthma.[50-52] Consistent with the pattern of the results of total serum IgE concentrations, methacholine airway responsiveness, and FEV_1 in boys, the prevalence of asthma was also lower in those boys exposed to 2 or more dogs or cats compared with those who were unexposed (5.1% vs 11.8%). This difference was not statistically significant across pet exposure categories; however, only 39 boys were exposed to 2 or more dogs or cats in the first year of life. Assuming prevalences that we found in our cohort, a study designed to detect a statistically significant difference in the prevalence of asthma among boys exposed to 2 or more dogs or cats would have required a final cohort of at least 1327 children followed up to age 6 to 7 years.

An important strength of this study is the prospective design using a population-based cohort of children followed yearly from birth. The prevalences of allergic sensitization, methacholine airway responsiveness, and asthma found in our cohort were similar to those reported by others studying children of similar ages.[53-55] The mean values for total serum IgE were also similar to those reported by others.[55,56] Animal exposure was ascertained when the child was 1 year old, not years later.[10,14,15] Assessing animal exposure prior to assessing outcomes reduces concern of misclassification of exposure and recall bias. Information on other factors potentially related to risk of allergic sensitization, most importantly family history, was collected, allowing adjustment for the potential confounding effects of these other variables.[37] Another strength is the multiple objectively measured outcomes.[10,14] The association between pet exposure and less allergic sensitization was found with both in vivo (skin prick test reactivity) and in vitro (allergen-specific IgE levels) tests. The per-

sons performing the skin prick tests, allergen-specific IgE tests, spirometry, and methacholine challenges were unaware of study hypotheses at the time the tests were performed, making systematic measurement bias unlikely.

Bronchial hyperresponsiveness is frequently stated to be a major component of asthma that can be objectively measured.[58-60] Our findings of reduced methacholine airway responsiveness in boys with exposure to 2 or more dogs or cats suggest that exposure to dogs and cats may be associated with a reduced risk of asthma, at least in boys. While we did not find a statistically significant association for current asthma in this study, the prevalence of asthma in boys exposed to 2 or more dogs or cats was 57% lower than in unexposed boys, a difference that would likely be significant in a larger population.

There are limitations to our study. As with most prospective studies, some children did not complete the entire study. However, we found no important differences between children examined at age 6 to 7 years and those who were not examined. In addition, we could not detect differences in the relationship between dog and cat ownership and parental history of asthma, allergy, or hay fever among those examined and not examined. A second caveat is the limited racial, socioeconomic, and geographic diversity of our study population, suggesting that our conclusions can only be applied to similar populations of white children. Since our follow-up was limited to an average age of 6.7 years, we do not know if the associations we found will persist as the children grow older, but others have found that the association between dog and cat exposure and a lower risk of allergy-related symptoms persisted to age 12 to 13 years.[17,44] Sample size is another limitation of the study. A larger sample would have allowed more reliable estimates and detailed examinations of the differences between boys and girls and between children with and without parental histories of asthma. A final caveat is that we did not consider exposure to dogs and cats outside the child's home.

In this prospective study designed to examine multiple potential risk factors for allergic sensitization, we found that exposure to 2 or more dogs or cats in the first year of life was associated with a significantly lower probability of subsequent allergic sensitization to common aeroallergens. Exposure to 2 or more dogs or cats was also associated with significantly lower serum IgE concentration, less methacholine airway responsiveness, and better lung function in boys but not in girls. The association between pet exposure and decreased prevalence of allergic sensitization remained unchanged after adjustment for potentially confounding variables. These findings suggest that exposure to more than 1 dog or cat in the first year of life may reduce a child's risk of allergic disease.

Author Contributions: *Study concept and design:* Ownby, Johnson.
Acquisition of data: Ownby, Johnson.
Analysis and interpretation of data: Ownby, Johnson, Peterson.
Drafting of the manuscript: Ownby, Johnson, Peterson.
Critical revision of the manuscript for important intellectual content: Ownby, Johnson, Peterson.
Statistical expertise: Peterson.
Obtained funding: Ownby, Johnson.
Administrative, technical, or material support: Ownby, Johnson.
Study supervision: Ownby, Johnson.
Funding/Support: This work was supported by grant AI 24156 from the National Institute of Allergic and Infectious Diseases, by the Fund for Henry Ford Hospital, and by grant PO3ES06639 from the National Institute of Environmental Health Sciences.

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that only about 2% of parents fall in this group, the rest are difficult to reach, apathetic or otherwise lacking in motivation. In many countries, the children of such parents are protected by herd immunity, and an interesting change in trends occurs when there is a brisk little epidemic caused by the immunization rate falling below a certain threshold (typically around 75%). For example in the case of whooping cough, a relatively small amount of publicity showing hospitalised, very sick cases will send the mothers and babies scurrying to the vaccination centre in quite a hurry! In this regard, we have not been worrying enough about booster doses following basic infant immunization. During the brisk diphtheria epidemic in the countries of the former USSR in 1995 and 1996, a significant proportion of the cases were in adults. Whereas many adults have tetanus boosters, e.g. when they injure themselves, few countries have active plans for regular diphtheria boosters.

Autoimmunity and immunization

Drs Shoenfeld and Aron-Maor have done an excellent job in summarizing the current literature on the possibilities of vaccines acting as a trigger for autoimmune phenomena. The story of hepatitis B vaccine and multiple sclerosis is most interesting. A very modest but nevertheless clear-cut and possibly statistically significant increase in demyelinating disease in adults immunized with hepatitis B vaccine has been noted in France, a country in which a very active whole-of-population campaign has been introduced. In the USA, on the other hand, no such association has been noted. United States authorities have concluded that perhaps their follow-up procedures are suboptimal. Demyelinating phenomena appear not to have been noted in infants or young children. As most countries now have hepatitis B immunization as part of the national plan in infancy, it well may be that this problem (if it is one) is self-solving. These authors conclude that the issue of the risk of vaccination remains a philosophical one, as the risks remain somewhat conjectural but the benefits represent a public health triumph of major dimensions. To this the present author would only add that the risk-benefit equation is tilted vastly in favour of immunization in the developing countries, where three-quarters of the deaths which occur in the under-14 age-group are due to communicable diseases. Of course, the problem at issue would disappear, disease by disease, if eradication attempts were to be successful. We have eradicated smallpox, are well on the way to eradicating poliomyelitis, and can envisage the possible eradication of measles were sufficient resources devoted to the problem. Many other disease could potentially be eradicated. On balance, this is the shining goal for 21st century preventive medicine.

Article No. jaut.1999.0349, available online at http://www.idealibrary.com on IDEAL®

Journal of Autoimmunity (2000) 14, 15–22

Stimulation of the Developing Immune System Can Prevent Autoimmunity

Bhagirath Singh

Department of Microbiology & Immunology, University of Western Ontario and John P. Robarts Research Institute, London, Ontario N6A 5C1, Canada

Key words: immunostimulation, immunoregulation, regulatory T cells, vaccination, infection, autoimmunity

Both genetic and environmental factors contribute to the development of autoimmunity. Animals and humans exposed to natural infections have a reduced rate of autoimmune diseases. There is increasing evidence that immune stimulation prevents autoimmune diseases. Our hypothesis is that the process of the development of pathogenic cells involved in autoimmunity can be modulated by early stimulation of the immune system in autoimmunity prone individuals This allows for the upregulation of cytokines and growth factors that influence the generation of regulatory cells involved in autoimmunity. As we live in a 'cleaner environment' the decreasing chances of natural infection in the general population may contribute to the induction of autoimmunity because the developing immune system is not exposed to stimulation that may be necessary to generate regulatory cells involved in the modulation and prevention of autoimmunity. Immunization with certain vaccines may provide an alternative approach to stimulate the immune system to modulate or prevent the generation of pathogenic cells involved in autoimmunity by induction of regulatory cells.

Correspondence to: Dr Bhagirath Singh, Dept. Microbiology and Immunology, University of Western Ontario, London, Ontario N6A 5C1, Canada. Fax: 519–661–3499. E-mail: bsingh@julian.uwo.ca

0896–8411/00/010015+08 $35.00/0

Introduction

A normal immune system develops through the interaction of many cellular and humoral components that develop at different rates during fetal and early postnatal life. Many cells involved in the immune response are derived from undifferentiated heamo poietic stem cells [1]. The selection and expansion of the specific lymphocytic repertoire that arises from these cells is influenced by both genetic and environmental factors [2]. In individuals genetically susceptible to autoimmune diseases these stem cells give rise to pathogenic cells [3]. Our hypothesis is that the development of pathogenic cells can be modulated by early vaccinations which allows for the upregulation of cytokines and growth factors that influence the generation of regulatory cells in autoimmunity prone individuals (Figure 1). The stimulation can be non-specific or antigen specific during the early development of the immune system. There probably is a window of opportunity when agents that stimulate the immune system can influence this process. Treatment before or after this time frame may not protect the development of autoimmune diseases.

Several observations (reviewed in 4) support the idea that microbial agents influence the occurrence or course of certain autoimmune diseases: i) there appears to be a North/South gradient that is correlated with higher autoimmune disease incidence in northern countries, ii) migrant populations acquire the autoimmunity prevalence of the areas to which they move, iii) more than 2/3 of identical twins are discordant for autoimmune diseases, iv) non-obese diabetic (NOD) mice, the inbred animal model of spontaneous Type I diabetes, show variable disease incidence in different colonies and are protected from disease by microbial infections [5].

Considerable evidence indicates that T cells mediate most autoimmune diseases. In most cases co-operation between $CD4^+$ and $CD8^+$ T cells is required to promote the development of autoimmunity [6]. Indirect evidence suggests that $CD4^+$ Th1 cells and $CD8^+$ cytotoxic T cells are the effector cells in most autoimmune diseases. The $CD4^+$ Th2 cells, which preferentially secrete IL-4 and Il-10, are the protective regulatory cells in autoimmunity [7]. Recently, in addition to $CD4^+$ Th2 cells [8] several other T cell subsets have been proposed to function as regulatory T cells. These include intestinal mucosa-derived $CD4^+$ Th3 cells which secrete IL-4, IL-10 and TGF-β [9]; IL-10 induced $CD4^+$ T regulatory type 1 (Tr1) cells [10], $CD4^+$ $CD25^+$ regulatory T cells [11] and $CD8^+$ $TCR\gamma\delta^+$ T cells [12]. Indeed *in vivo* upregulation of such regulatory T cells by adjuvants [13–15], autoantigens [16–19] or dendritic cells [20, 21] prevents type I diabetes.

The NK-T cells also appear to play a strong regulatory role in autoimmunity [22]. In humans and NOD mice, a decreased frequency of IL-4 producing NK-T cells and impaired IL-4 production by peripheral blood T cells is correlated with increased susceptibility to type I diabetes [23–25]. Therefore, a relative absence of IL-4 producing NK-T cells may alter the Th1/Th2 cell balance, thereby establishing an extreme Th1 phenotype and initiating events that lead to the onset of type I diabetes [23, 25].

Strong evidence exists for the presence of regulatory $CD4^+$ T cells in prediabetic NOD mice. Adoptive transfer of type I diabetes can be prevented by cotransfer of $CD4^+$ T cells from young non-diabetic mice [26, 27]. The absence of regulatory $CD4^+$ T cell function at an early age allows for the induction of pathogenic islet-reactive T cells and trigger the development of insulitis and type I diabetes [28]. Several therapies prevent diabetes in NOD mice during the early phase of the development of the immune system because they may promote the development of regulatory cells [4, 29].

Various environmental factors including microbial infections, dietary components and seasonal variation influence the development of disease [30–32]. Interestingly, there are definitive differences in the incidence of type 1 diabetes between different countries with Scandinavian countries [33] having the highest relative incidence (>30/100,000 each year) suggesting that there may be some inverse association between the development of diabetes and environment and socio-economic conditions. One of the important links between these observations and type I diabetes onset is the role that immune system may play. Given the millions of years of co-evolution of our immune system with the microbial world, it is not surprising that there could be a direct influence of microbial agents on autoimmune diseases. Many environmental factors can influence the immune system that eventually controls the induction and progression of type I diabetes and other autoimmune diseases [4]. Cross reactivity between microbial antigens and autoantigens [34], the immunoregulatory properties of viral proteins and the production of cytokines or chemokines due to microbial infection could be responsible for the induction or protection from disease [35, 36].

Microbial infections have the capacity to regulate autoimmunity both positively and negatively [31]. NOD mouse colonies around the world have a wide range of diabetes incidence reflecting the environmental effects with a greater incidence of diabetes in those colonies tested to be specific pathogen free [37]. The disease incidence appears to be between 20–90% in females at 300 days, while males have an incidence of 1–65% [38]. There appears to be a reciprocal relationship between the incidence of diabetes and the level of microbial infection within the NOD mouse colonies [32].

Immunostimulation prevents autoimmunity

We postulate that decreasing natural infections contribute to the development of autoimmunity because the developing immune system is not exposed to

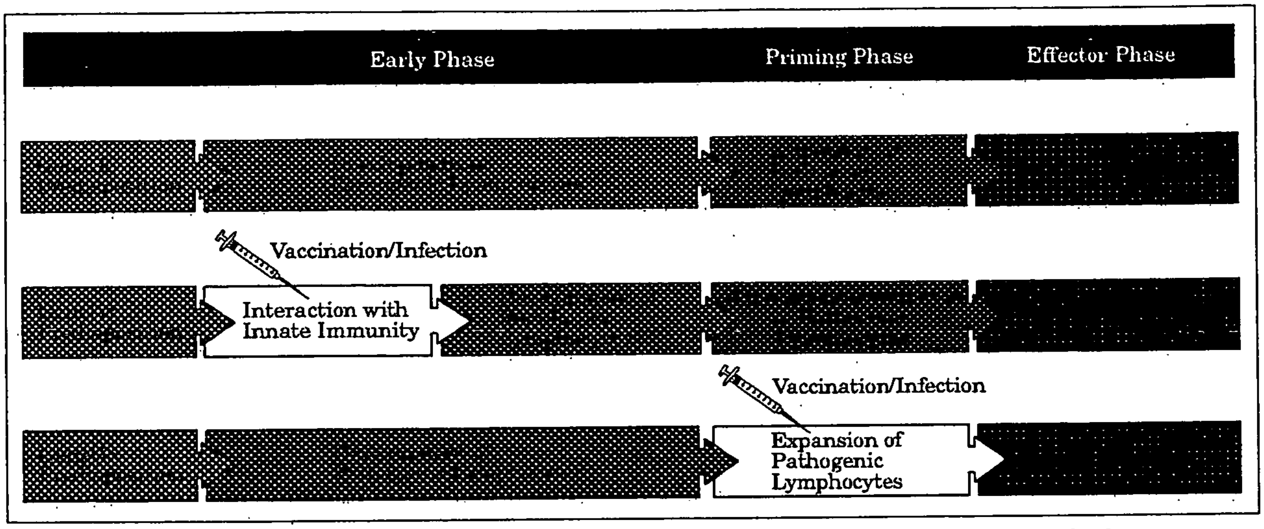

Figure 1. Immunostimulation of the developing immune system in genetically susceptible individuals can prevent the development of autoimmunity by inducing regulatory T cells.

stimulation that may be necessary for the generation of regulatory cells involved in the prevention and modulation of autoimmunity. Immunization with vaccines provides an artificial way to stimulate the immune system that could modulate or prevent the generation of pathogenic cells involved in autoimmunity. Immunostimulation by various treatments have been shown to prevent autoimmunity in animal models [30, 31, 39]. We have reported that a single injection of Bacillus Calmette Guerin (BCG, containing *Mycobacterium bovis*) or complete Freund's adjuvant (CFA, containing *Mycobacterium tuberculosis*) was highly effective in inducing long term protection from type I diabetes in NOD mice [13, 14] and BB rats [40]. Pretreatment with adjuvants containing mycobacterial preparations such as CFA prevented the induction of experimental autoimmune encephalomyelitis (EAE) in susceptible animals [41]. Thus, immune response to microbial antigens (pathogens) could prevent autoimmunity [30]. Once the pathogenic cells are activated such as development of insulitis in NOD mice, the CFA treatment could not block the progression to type I diabetes [14, 42]. Although treatment with BCG or CFA is also effective in blocking the recurrence of disease in syngeneic islet transplanted NOD mice [43, 44], the mechanism is likely to be different for disease prevention [45]. The protection of NOD mice by adjuvants is associated with the production of protective cytokines such as IL-4 [46, 47], TGF-β and the down regulation of Fas expression in the islets of treated animals [48].

Based on the above studies several clinical trials using BCG vaccination have been carried out to block the progression of type I diabetes [49–54]. The first trial in newly diagnosed type I diabetic patients showed a 65% remission in the vaccinated group and 7% in the controls [49]. However, a number of recent clinical trials and prospective studies have not supported these earlier results [50–54].

Our recent studies in NOD mice suggest that the window of opportunity for protection from type I diabetes following disease induction is relatively narrow [42]. We found that Cyclophosphamide (CY) accelerated type I diabetes in young NOD mice can be prevented by BCG vaccination. However, to be effective BCG must be given within 3 days of CY treatment [42]. If BCG therapy was delayed until day 7 following CY treatment, none of the animals were protected from type I diabetes. One possible explanation of our data is that once intra islet insulitis is established by primed cells, treatment with BCG is much less likely to be successful to prevent type I diabetes [54]. This probably is also the reason why older animals with no clinical disease but with significant insulitis cannot be protected from diabetes by immunostimulation [14] (Figure 1). Moreover repeated BCG immunization appears to be more effective than a single dose in preventing diabetes in NOD mice [55]. A point that may be critical for human clinical trials. Although BCG reduces the incidence of diabetes in prediabetic NOD mice and BB rats, it has no beneficial effect given after disease onset similar to what has been found in human clinical trials. Perhaps the window of opportunity to prevent disease in humans may be similar to NOD mice therefore in BCG therapy has to be administrated before the onset of insulitis [56].

Infections in early life may be associated with a reduced risk of type I diabetes in humans [57]. Recent epidemiological data imply that BCG vaccination performed neonatally may be associated with lower risk of type I diabetes than later vaccination [58]. A case-control study showed the relative risk of developing type 1 diabetes to be reduced in children vaccinated against measles [59]. Immunization with some common pediatric vaccines may therefore have an important protective role in the development of type I diabetes.

Immunization with microbial agents and development of autoimmunity

The idea that autoimmune diseases can be precipitated by microbial pathogens was proposed more than a century ago (reviewed in 37). In particular rubella, Coxsackie and cytomegalovirus infections have been linked to type I diabetes. At least in one case, the virus was isolated from pancreas at the time of autopsy and in others, anti-viral antibodies were detected in the sera at the time of diagnosis [60]. If viral infections cause autoimmune diseases, there is a possibility that vaccination may induce autoimmunity, especially when live, attenuated virus is used, as in measles, mumps, and rubella (MMR) vaccination. However, previous exposure to measles, mumps, and rubella (MMR) by natural infection or vaccination or by new immunization with MMR vaccine, changed neither the prevalence nor the level of autoantibodies against thyroid cell or pancreatic islet β-cell antigens (ICA) [61]. Thyroid autoantibody levels and prevalence were lower in children with antibodies against measles, mumps, or both before vaccination than in children without those antibodies. However, children with rubella antibodies before vaccination had higher levels of ICA than did the rubella seronegative individuals. More recently viral vaccines have been linked to autoimmune conditions such as pediatric asthma [62] and inflammatory bowel disease [63]. However, there is no conclusive evidence that type I diabetes in humans results from a viral infection.

It has been suggested that immunization after the age of two months is associated with an increased risk of diabetes mellitus in children [64]. The vaccine for *Haemophilus influenzae* type b has also been specifically implicated as having this potential [58]. Dahlquist *et al.* examined the effect of vaccination on the incidence of type I diabetes [59]. No single vaccine, or combination of vaccines, was linked to an increase in diabetes, and children who had received a measles vaccine had a decreased risk of type I diabetes. Another study of the possible link between immunization with vaccines and type I diabetes did not provide any new insight [65]. A recent study from Finland found no statistical difference in the cumulative incidence of diabetes at the age of 10 years between children vaccinated with *H. influenzae* at various ages [66]. The authors concluded that: i) the vaccination programs are not responsible for the increase in type I diabetes, ii) there is no difference in the risk of type 1 diabetes between children not vaccinated against *H. influenzae* type b and those vaccinated at the age of 24 months only and iii) the difference in risk between children vaccinated against *H. influenzae* type b at the age of 3 months and those vaccinated at the age of 24 months was not statistically significant. The specific role of immunostimulation by vaccination was recently found with enterovirus (polio) vaccine. Children who develop strong immune response to this vaccine were protected from diabetes [67].

In another study no difference was found in the development of β-cell autoimmunity or type I diabetes between diabetics and controls that were vaccinated with hepatitis B, *Haemophilus influenzae b*, polio or diphtheria tetanus pertussis (DTP) vaccines [68]. This suggests that early childhood immunization with common vaccines does not seem to increase the risk of developing diabetes. In fact it may decrease the risk [59, 64]. However, there is still need for long term cohort studies to examine the relationship between vaccines, their cross-reactivity and their ability to induce or protect from autoimmunity.

Mechanisms by which immunostimulation may prevent autoimmunity

Many studies have supported the concept that molecular mimicry, a process of antigenic cross-reactivity resulting from similarity in amino acid sequence or structure, could be one pathway by which autoimmunity is induced or modulated by microbial pathogens [69]. There is evidence linking the β-cell autoantigens in diabetes with microbial epitopes through molecular mimicry [34]. Cross-reactive epitopes may be generated by antigen processing, which may be presented by host MHC molecules to autoreactive T cells [31]. However, early immune responses to self proteins in NOD mice appear to be towards several autoantigens, including glutamic acid decarboxylase (GAD), insulin, heat shock protein 60 and carboxypeptidase H [70]. Subsequently, multiple GAD T cell epitopes are recognized at the time of β-cell injury by T cells [71]. The autoreactive T cell responses in NOD mice may represent both intra-molecular and inter-molecular spreading of auto-reactivity with age and leading to type I diabetes [70, 72, 73].

There may be cross-reactivity between Rubella virus capsid protein and a 52 Kd islet antigen [74]. There is sequence homology between human GAD65 and Coxsackie B virus P2-C protein, which contains a T cell epitope involved in GAD responses in humans with type I diabetes [75]. T cell responses in newly diagnosed type I diabetes patients directed against amino acid regions 247–266 and 260–279 of P2-C obviously overlap with the 250–273 region of GAD which contains sequence homology [75]. Furthermore, immunization of NOD mice with Coxsackie virus P2-C protein can induce T cell responses in the mice that cross react with GAD or GAD peptides [76]. Despite these studies, there is no direct evidence for the involvement of molecular mimicry in autoimmune diseases [77].

Microbial products are likely involved in the activation of CD4 Th1 or Th2 cells and/or CD8 cytotoxic T cells (CTL) cells in autoimmunity. The microbial products could have a bystander effect on the T cells through the activation of monocytes, dendritic cells and other non lymphoid cells (basophils, eosinophils etc.) which produce various regulatory cytokines. Production of IL-12 has been shown to be involved in Th1 cell activation while IL-4 induces the activation of Th2

cells [78]. Microbial products induce the production of IL-12 by the mononuclear cells [79]. In NOD mice there is strong evidence that Th1 cells producing IFN-γ and IL-2 are the diabetogenic cells where as the Th2 cells which produce IL-4, IL-10 etc. are at least non pathogenic or even protective. It has been found that the administration of IFN-γ accelerates the disease while IL-4 is protective in spontaneous disease, further supporting the above conclusions [4].

Activation of regulatory T cells in response to microbial infection (Figure 1) will depend upon many factors including i) the nature of cytokines produced by the innate immune system in the host by the infection, ii) cross-reactivity of microbial epitopes with the autoantigens, iii) density and affinity of these epitopes for MHC molecules. As shown in the Figure 1 viral infection of the islets could lead to the release of self antigens from the β-cells. We have previously shown that cross-reactive epitopes with strong MHC binding affinity induces the activation of Th1 responses where as low affinity epitopes result in the activation of Th2 responses of same antigen specificity [80]. Microbial infection would result in the generation of epitopes with different affinities for MHC molecules, some of which may cross-react with the islet antigens. This would result in the activation of Th1 or Th2 cells. High viral dose or repeated viral infection could result in Th1 responses and mild infection could result in Th2 responses. Therefore certain viruses may prevent or induce autoimmunity in susceptible subjects. The direct role of Th1 and Th2 cells in autoimmunity remains unclear as both types of cells can transfer disease on their own [81]. In spontaneous disease CD8 CTL are also required and their activation requires the Th1 cells [4].

The alternative concept of bystander activation proposes [77] that immunostimulation induces regulatory T cells by attracting and activating antigen-presenting cells; or by perturbing the cytokine balance through the inflammation that is associated with infection. Potentially, bystander autoimmunity may fall within the broader concept of innate immunity inducing cells that produce various regulatory cytokines that in turn induce protective or pathogenic cells (Figure 1).

Implications for the therapy of autoimmunity

The above discussion suggests a strong pathogenic or preventative role for microbial agents in autoimmune diseases. Many bacterial agents such as BCG vaccine offer a rational approach for the prevention of autoimmune diseases [30, 31]. Despite their strong diabetes protective effect in NOD mice and BB rats these approaches have not been very effective in children after the disease onset [49–54]. Therefore, much work needs to be done to determine the dose, time, and route of administration of these therapies in human [56, 82]. The oral or intranasal administration of autoantigens such as insulin, IA-2, GAD or their immunodominant epitopes in conjugation with microbial agents may offer powerful new approaches to prevent autoimmune diseases through the mucosal immune system [83–85]. We believe that microbial products or autoantigens capable of inducing such immunoregulatory responses may provide alternatives to immune suppression for prevention of autoimmunity in subjects at high risk for developing disease. These studies may also be useful for the prevention of recurrence of autoimmunity [31].

Any therapy to prevent autoimmunity should be done with risk-benefit considerations. The use of experimental models of autoimmunity such, as the NOD mice are a good alternative to preclinical trials in humans. Our results suggest that the window of opportunity for the prevention of autoimmunity or its progression by stimulation of the immune system is relatively narrow [42, 56]. An optimal stimulation may be necessary and not all vaccines or therapies may be effective in disease prevention. What it means in practical terms is that careful consideration should be given to carrying out prevention trials with appropriate agents in the early phase of the development of the disease where immunostimulation is more likely to be successful in inducing regulatory T cells that prevent autoimmunity.

Acknowledgements

I thank Sean Prange for the preparation of the figure and Tracey Stephens for helpful comments on the manuscript. The work in our laboratory is supported by grants from the Juvenile Diabetes Foundation, the Canadian Diabetes Association and the Medical Research Council of Canada.

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